BOX PCT

ATTORNEY'S DOCKET NO: 24498

U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE		DATE: January 2001 (18.01.2001)
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLN. NO. (if known): Not Yet Assigned 09/744016
INTERNATIONAL APPLICATION NO.: PCT/EP99/05220	INTERNATIONAL FILING DATE: 22 July 1999 (22.07.99)	PRIORITY DATE CLAIMED: 22 July 1998 (22.07.98)

TITLE OF INVENTION: **METHOD FOR SCREENING OF MODULATORS OF CALCINERUIN ACTIVITY**

APPLICANT(S) FOR DO/EO/US: VOELKEL, Helge

Applicant hereby submits to the Unites States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. __ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. X This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

4. X A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. x A copy of the International Application as filed (35 U.S.C. 371(c)(2)):

   a. X is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ has been transmitted by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. X A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. X Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

   a. __ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. __ have been transmitted by the International Bureau.
   c. __ have not been made; however, the time limit for making such amendments has NOT expired.
   d. X have not been made and will not be made.

8. __ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. X An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. X A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11. TO 16. BELOW CONCERN OTHER DOCUMENT(S) OR INFORMATION INCLUDED:

11. ___ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. X An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. X A **FIRST** preliminary amendment.
    __ A **SECOND** or **SUBSEQUENT** preliminary amendment

14. __ A substitute specification.

15. __ A change of power of attorney and/or address letter.

16. X TRANSMITTAL FORM; FEE CALCULATION; INTERNATIONAL PUBLICATION WO 00/05363; INTERNATIONAL PUBLICATION DATE 03 FEBRUARY 2000 WITH ENGLISH TRANSLATION CONSISTING OF 130 PAGES INCLUDING; 1COVER PAGE CONTAINING THE ABSTRACT; 42 PAGES TEXTUAL SPECIFICATION, 4 PAGES OF 23 CLAIMS; 0 SHEETS DRAWINGS; 83 PAGES SEQUENCE LISTING; PCT/ISA/210 INTERNATIONAL SEARCH REPORT; PCT/IPEA/409 INTERNATIONAL PRELIMINARY EXAMINATION REPORT WITH AMENDED SHEETS (CLAIMS) TO BE EXAMINED; PRELIMINARY AMENDMENT TO IPER AMENDED SHEETS; UNEXECUTED INVENTOR'S DECLARATION; ASSIGNMENT AND RECORDATION COVER SHEET; PCT/RO/101 REQUEST; PCT/IB/304 NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENT; PCT/IB/332 INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION ; PCT IPEA/402 NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY.

ATTORNEY'S DOCKET NO: 24498

U.S. APPLICATION NO. (if known) not yet assigned 09/744016	INTERNATIONAL APPLICATION NO. PCT/EP99/05220	DATE: January 2001 (18 .01.2001)

17. x The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO:.....$860.00	$ 860.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482)..... .....$690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))..... $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... $1000.00		
International preliminary examination fee (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NO. FILED	NO. EXTRA	RATE		
TOTAL	23 -20=	3	X $ 18.00	$ **54.00**	
INDEPENDENT	2 - 3=	1	X $ 80 00	$ 0.00	
Multiple dependent claims(s) (if applicable)			+ $260.00	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ **914.00**	
Reduction by ½ for asserting small entity, if applicable. (Note 37 CFR 1.9, 1.27, 1.28).				$ **457.00**	
SUBTOTAL =				$ 457.00	
Processing fee of **$130.00** for furnishing the English translation later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
TOTAL NATIONAL FEE =				$ 457.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property +**				$ **40.00**	
TOTAL FEES ENCLOSED =				$ **497.00**	
				Amount to be: refunded	$
				charged	$

ATTORNEY'S DOCKET NO: 24498

U.S. APPLICATION NO. (if known) not yet assigned	INTERNATIONAL APPLICATION NO. PCT/EP99/05220	DATE: January 2001 (18.01.2001)

09/744016

a. X Two checks in the amount of $497.00 to cover the above fees are enclosed.

b. ___ Please charge my Deposit Account No. 14-0112 in the amount of $_____ to cover the above fees. (A duplicate copy of this sheet is enclosed.)

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0112.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed to request that the application be restored to pending status.

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Rev. 02/98

BOX PCT

Attorney Docket No. 4498

09/744016

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

JC07 Rec'd PCT/PTO 19 JAN 2001

In re Application of:
VOELKEL, Helge
International Application No. PCT/EP99/05220
Serial No. NOT YET ASSIGNED
International Filing Date: 22 July 1999 (22.07.99)
Filed: January 19, 2001
For: **METHOD FOR SCREENING OF MODULATORS OF CALCINEURIN ACTIVITY**

TRANSMITTAL LETTER

Commissioner of Patents
Washington, D.C. 20231
Sir:

Submitted herewith for filing in the U.S. Patent and Trademark Office is the following:

(1) Transmittal Letter
(2) Transmittal Letter To U.S. Designated/Elected Office (DO/EO/US) Concerning Filing under 35 U.S.C. 371
(3) International Publication No: WO 00/05363
International Publication Date: 3 February 2000 (03.02.00)
which consists of 130 pages including:
1 cover sheet containing the Abstract
42 pages Textual Specification
4 Pages of 23 claims
0 Sheets of Drawings
83 pages Sequence Listing
(4) PCT/ISA/210 International Search Report
(5) PCT/IPEA/409 International Preliminary Examination Report with Amended Sheets (claims) to be examined
(6) Preliminary Amendment to IPER Amended Sheets
(7) Unexecuted Inventor's Declaration
(8) Assignment and Recordation Cover Sheet
(9) PCT/RO/101 PCT Request
(10) PCT/IB/304 Notification Concerning Submission of Priority Document
(11) PCT/IB/332 Information Concerning Elected Offices Notified Cf Their Election
(12) PCT/IPEA/402 Notification of Receipt of Demand by competent International Preliminary Examining Authority
(13) Check No. 14086 $457 .00 for Government Filing Fee as a small entity
(14) Check No. 14087 $ 40.00 for recordation fee
(15) Postcard for early notification of serial number.

Respectfully submitted,
NATH & ASSOCIATES PLLC

By: _____
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PCT/EP99/05220 19 JAN 2001

#4

BOX PCT-MISSING REQUIREMENTS
Attorney Docket No. 24498

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
VOELKEL, Helge
International Application No. PCT/EP99/05220
Serial No. 09/744,016
International Filing Date: 22 July 1999 (22.07.99)
Filed: January 19, 2001
For: **METHOD FOR SCREENING OF MODULATORS OF CALCINEURIN ACTIVITY**

SUBMISSION OF MISSING REQUIREMENTS

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements Under 35 U.S.C. 371 mailed 9 March 2001, submitted herewith are the following:

(1) Executed Declaration and Power of Attorney
(2) Computer readable Form of the Sequence Listing
(3) Verified Statement Claiming Small Entity Status
(4) Two (2) Forms PCT/IB/306 Notification of the Recording of a Change
(5) Check No. 14681 $ 65.00 surcharge for late filing of Declaration as a small entity.

The Computer Readable Copy of the sequence listing is being submitted herewith on a 3.5" computer disk. As required by 37 CFR § 1.821(f), the Applicants hereby submit that the sequence information recorded on the enclosed computer disk is identical to the paper copy of the listings in the specification and includes no new matter.

The Commissioner is hereby authorized to charge any deficiency or credit any excess to Deposit Account No. 14-0112.

Respectfully submitted,
NATH & ASSOCIATES PLLC

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BOX PCT
Attorney Docket No. 24498

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
VOELKEL, Helge
International Application No. PCT/EP99/05220
Serial No. NOT YET ASSIGNED
International Filing Date: 22 July 1999 (22.07.99)
Filed: January 19, 2001
For: **METHOD FOR SCREENING OF MODULATORS OF CALCINEURIN ACTIVITY**

PRELIMINARY AMENDMENT

Commissioner of Patents
Washington, D.C. 20231

Sir:

Before calculating the filing fee for the above identified application, please enter the following amendments:

IN THE CLAIMS:

Claim 3, line 1, delete "or 2".

Claim 4, line 1, delete "or 2".

Claim 5, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 6, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 8, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 9, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 10, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 13, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 14, line 1, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 17, line 1, delete "or 16"

Claim 18, line 1, delete "one of claims 3 to 17" and insert in lieu thereof --claim 3--.

Claim 19, line 2-3, delete "one of the preceding claims" and insert lieu thereof --claim 1--.

Claim 21, line 1, delete "or claim 20"

Claim 22, line 1, delete "one of claims 19 to 21" and insert lieu thereof --claim 19--.

REMARKS

The above amendments have been made to remove multiple dependencies from the claims, and no new matter has been added.

Respectfully submitted,

NATH & ASSOCIATES PLLC

By: _____

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PCT09

RAW SEQUENCE LISTING DATE: 02/14/2002
PATENT APPLICATION: US/09/744,016A TIME: 10:12:44

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Method for screening of modulators of calcineurin activity

Specification:

The invention relates to a method for screening of modulators of calcineurin.

Calcineurin (E.C. 3.1.3.16) is a serine/threonine phosphoprotein phosphatase and is composed of a catalytic (calcineurin A) and regulatory (calcineurin B) subunit (about 60 and about 18 kDa, respectively). In mammals, three distinct genes (A-alpha, A-beta, A-gamma) for the catalytic subunit have been characterized, each of which can undergo alternative splicing to yield additional variants. Although mRNA for all three genes appears to be expressed in most tissues, two isoforms (A-alpha and A-beta) are most predominant in brain.

Calcineurin has been cloned from various organisms including human (Guerini et al., 1989), (Guerini and Klee, 1989), (Kincaid et al., 1991), (Kuno et al., 1989), (Ito et al., 1989), (Muramatsu and Kincaid, 1993). The crystal structure has shown that calcineurin A contains a binuclear metal center with unknown enzymatic function (Griffith et al., 1995). Recombinant expression of rat calcineurin A subunit in bacteria or SF9-cells were not effective and yielded only poor enzymatic activities since calcineurin A is not stable in the absence of calcineurin B (Perrino et al., 1992), (Perrino et al., 1995), (Haddy and Rusnack, 1994). Coexpression of calcineurin A and calcium binding subunit calcineurin B yielded a more stable and active enzyme (Mondragon et al.,

1997). Calcineurin has been implicated in various neuronal signaling pathways (Klee et al., 1988), (Yakel, 1997) but the neuronal function is only poorly understood (Guerini, 1997).

Calcineurin is the only protein phosphatase known to be under the control of Ca^{2+} and calmodulin. Binding of Ca^{2+} and calmodulin is necessary for enzymatic activity. Calmodulin is bound by the catalytic subunit whereas the regulatory subunit possesses four Ca^{2+} binding sites.

Calcineurin is discussed in the context of immunosuppression. It has been shown that calcineurin acts via the transcription factor NFAT (nuclear factor of activated T cells) on the T cell response. The functions of NFAT proteins are directly controlled by calcineurin in a calcium- and calmodulin-dependent manner. Activation of NFAT by calcineurin is mediated by the cytosolic binding protein FKBP.

Substances which are able to block the calcineurin signal pathway are suitable agents in order to block the T cell activation and thereby suppressing the immune response. Suppression of immune response has important clinical relevance, for example in transplantation surgery for preventing rejection episodes. Therefore, calcineurin as pharmacological target is of great importance and several attemps were made to develop agents which block the calcineurin signal pathway. Examples of such immunosuppressive drugs are FK506 (Fujisawa) and cyclosporine (Novartis) (Liu et al, 1991). These antibiotics inhibit calcineurin phosphatase activity in the presence of immunophilin receptor proteins (FKBP, cyclophillin) and thereby suppress immune response by preventing the activation of the T cell transcription factor NFAT (Liu et al., 1992), (Nelson et al., 1993). FK506 (tacrolimus) binds to the binding protein FKBP and thereby prevents calcineurin

from binding to FKBP. Accordingly the signal pathway is interrupted. No activation of the transcription factor NFAT is achieved and the T cell activation is disturbed.

Nevertheless, there are several severe disadvantages and side-effect of said drugs. In clinical trials with liver and renal transplant recipients it has been shown that FK506-based therapy was associated with increased toxicities in comparison to conventional therapy. Furthermore FK506 has negative effects on the bone mineral physiology.

Besides the role of the calcineurin signal pathway in immune response it has been shown that calcineurin is involved in apoptosis induction by glutamate excitotoxicity in neuronal cells (Ankarcrona et al., 1996). Low enzymatic levels of calcineurin have been associated with Alzheimers disease (Ladner et al., 1996), (Kayyali et al., 1997). Calcineurin inhibitors (FK506, Cyclosporin) prevented epileptogenesis in model organisms (Moriwaki et al., 1996). In the heart or in the brain calcineurin also plays a key role in the stress response after hypoxia or ischemia (Butcher et al., 1997), (Hashimoto et al., 1998), (Molkentin et al., 1998).

In summary, calcineurin is a crucial target to develop new substances suitable as drugs, especially as immunosuppressive drugs. Former screening systems using purified calcineurin and conventional assays like radioactive or HPLC assays (Klee, 1991), (Enz et al., 1994) did not lead to appropriate new substances. Therefore, the invention has the object to provide a new screening system for modulators of calcineurin taking advantage of new insights into the signal pathway of calcineurin. By the use of this new screening system it is possible to develop new pharmaceuticals with respect to the field of transplantation surgery, cardiac infarction and

apoplexy, chronic or acute neurodegeneration and inflammatory diseases, for example. This object is solved by a method according to claim 1. Preferred embodiments of the inventive method are depicted in the dependent claims 2 to 17. A kit, vectors, cells and a peptide suitable for performing the inventive method are claimed in claim 18 to 23. The wording of all claims is hereby made to the content of the specification by reference.

The inventive method is based on results showing that a physiological interaction between calcineurin and superoxide dismutase takes place which provides a suitable target for developing of a new screening system.

For a long time it was not understood why recombinant or even purified calcineurin exhibited only 1 to 2 % of the specific activity estimated in crude brain extracts until it was detected that the binuclear metal center of the enzyme contains a redoxsensitive Fe^{2+} (Yu et al., 1997). After calcium activation or during purification procedure the Fe^{2+} is oxidized by oxygen species and turns the enzyme inactive (Stemmer et al., 1995), (Wang et al., 1996).

Recently it has been shown that copper/zinc superoxide dismutase (CuZnSOD, EC 1.15.1.1) protects calcineurin against oxidative inactivation (Wang et al., 1996). The phosphatase activity of calcineurin is strongly dependent on the presence of calcium and calmodulin. The addition of Ca^{2+} in the presence of calmodulin leads to a drastic increase in activity. But during several minutes this activity is lost. By the addition of copper/zinc superoxide dismutase the activity can be maintained.

Superoxide dismutase (SOD) dismutates the hyperoxide anion (superoxide) into hydroperoxide and molecular oxygen. There are two forms of this enzyme: the mitochondrial form containing manganese and the cytosolic form containing copper and zinc. In general superoxide dismutase is considered to be a catcher of radicals and is discussed in the field of detoxification of reactive oxygen species. Therefore, the role of superoxide dismutase in the protection of the activity of calcineurin found by Wang et al. was considered to be the result of general redox function of superoxide dismutase. Now, surprising results of the inventor lead to the knowledge that a physiological interaction between calcineurin and superoxide dismutase takes place. Several mutants of copper/zinc superoxide dismutase lacking the enzymatic function showed the protective effect on the activity of calcineurin. That means that the effect of CuZnSOD is not due to the function of superoxide dismutase in redox regulation. These results teach that superoxide dismutase interacts physiologically with calcineurin and that CuZnSOD is one component of the calcineurin pathway which is important for the physiological functions of calcineurin.

These results are used to develop a new screening system for modulators of calcineurin in order to find inhibitors or activators of the calcineurin signal pathway. The inventive method is based on the complex formation between calcineurin and superoxide dismutase in the presence of potential modulators of this physiological interaction. If a potential modulator disturbs the complex formation, this substance is a good canditate for inhibiting the calcineurin signal pathway and could possibly be used as immunosuppressive drug, for example. On the other hand it could be favourable to identify a substance which promotes complex formation and thereby stimulates the calcineurin signal pathway, e.g. the T cell

response in result. Such a substance could be used in order to strengthen immune response. By the term "modulator" is meant any substance which influences the complex formation relating to the inventive method. Additionally is meant any substance which influences the interaction between calcineurin and its substrates, e.g. the peptide RII. Furthermore is meant any substance which influences the superoxide dismutase and/or calcineurin on the transcriptional, the translational and/or the posttranslational level.

Calcineurin as used in the inventive method is build up by the regulatory subunit A and the catalytic subunit B. The presence of both subunits is essential for physiological activity of calcineurin. Nevertheless, it is possible to perform the inventive method using only one of the subunits. There are several isoforms of calcineurin consisting of subunit calcineurin B and one out of the group comprising subunit calcineurin A-alpha, A-beta and A-gamma. Each isoform represents a special cell and tissue specific distribution. Therefore, the choice of isoform could be crucial for cell and tissue specifity of the substance to be screened. With respect to clinical application of the substances to be screened preferably human forms of the proteins are used.

Furthermore it is preferred to perform the inventive method in the presence of calmodulin and calcium, because the activity of calcineurin is dependent on these factors. Preferably the cytosolic form of superoxide dismutase containing copper and zinc is used for complex formation, because interaction between the mitochondrial form of superoxide dismutase containing e.g. manganese normally does not occur under physiological conditions. The complex formation is performed in the presence of at least one potential modulator of calcineurin or the calcineurin signal pathway, respec-

tively. The complex comprising calcineurin A, calcineurin B, superoxide dismutase and preferably calmodulin is the target for potential modulators which could stabilize or disturb the complex.

Advantageously, the complex formation is monitored during the whole process. It is possible to add the modulator before or after the complex formation has been performed. Preferably the modulator is added before complex formation because the effect of a weak modulator will possibly not be monitorable when complex formation has already finished.

In principle, there are two possibilities to monitor the complex formation. Firstly, the complex formation is directly monitored by the use of labeled components in the complex, preferably by fluorescence detection. Secondly, the complex formation is monitored by the activity of the complex, especially the enzymatic activity of calcineurin. This second method can be performed in addition to the firstly mentioned method or as an alternative. Clearly, the inventive method is not restricted by the method for detecting the influence of the modulator on complex formation.

In a preferred embodiment of the invention, a mixture of substances comprising at least one potential modulator is analyzed by the inventive method. By isolating the complex together with the possibly interacting modulator it is possible to separate the modulator out of the mixture and to identify it by common methods.

In one preferred embodiment of the invention the calcineurin and/or superoxide dismutase are labeled. Especially preferred is the use of fluorescent labels. Preferably, the labeled proteins are fusion proteins comprising a fluorescent prote-

in, e.g. enhanced green fluorescent protein (EGFP). These fusion proteins are provided by genetic engineering methods. It is also possible to label said proteins by other methods known to experts in the art, e.g. by the use of radioactive isotopes which are incorporated into the proteins.

Advantageously the components of the complex, i.e. calcineurin and superoxide dismutase are expressed in the cell, especially in an eukaryotic cell, as fluorescent fusion proteins. By the use of laser fluctuation correlation spectroscopy the complex formation of labeled proteins is monitored directly within the cell. This embodiment of the invention is described in greater detail in the example. The invention comprises several vectors useful for the expression of calcineurin and/or superoxide dismutase in eukaryotic cells. These vectors encode the proteins, especially CuZnSOD and the different subunits of calcineurin, as fusion proteins in connection with the fluorescent protein EGFP (enhanced green fluorescent protein). EGFP is only one example of possible labels useful in respect of the inventive method. Furthermore, the invention comprises cells, especially eukaryotic cells, stably transfected with the above-mentioned vectors thereby expressing superoxide dismutase and/or calcineurin. Preferably, these proteins are coexpressed, i.e. expressed within the same cell.

In an especially preferred embodiment, the genetic information of fusion proteins is integrated in the cell by homologous recombination. That means that the gene encoding the recombinant protein, especially the fluorescent fusion protein, is incorporated in the genome of the cell in the place of the naturally occuring gene. This leads to a cell essentially lacking the natural protein. By the use of such cells it is possible to identify modulators by the inventive method which

influence the transcriptional, translational or posttranslation level of calcineurin and/or superoxide dismutase expression.

In another embodiment of the inventive method the components of the complex are isolated and preferably purified before complex formation is performed in vitro. Advantageously, the proteins are provided with a tag in order to facilitate purification, e.g. a histidin (his) tag consisting of several histidines in sequence which permits affinity purification by known procedures. Corresponding vectors encoding the tagged proteins are comprised by the invention. These vectors are especially useful as prokaryotic expression vectors. Furthermore, the invention comprises cells bearing said vectors.

Advantageously, following purification of the his-tagged proteins the tag is excised by appropriate enzymatic digestion, e.g. by the use of cathepsin-C of carboxypeptidase-A. Especially preferred is the purification of calcineurin by ferro-nitrilotricacetat-metal (Fe-NTA) affinity chromatography and the purification of superoxide dimutase by copper/-zinc-nitrilotriacetat-metal (CuZn-NTA) affinity chromatography. Nevertheless, other purification procedures known to experts in the art are possible. Natural occuring protein could also be used in the inventive manner.

Besides the use in purification of calcineurin and/or superoxide dismutase Ni (nickel)-NTA, Fe-NTA and/or Cu/Zn-NTA is used to immobilize the his-tagged calcineurin and/or superoxide dismutase in order to isolate naturally occuring ligands of these proteins using this inventive matrix. By the term "ligand" is meant any low- or highmolecular endogenous, exogenous or synthetic substance which interacts with said proteins. This could be a peptide, protein, carbohydrate,

lipid, nucleic acid or a synthetic polymer, for example. These so-identified ligands are potential candidates for modulators of the calcineurin signal pathway.

When performing complex formation in vitro it could be preferred to add calmodulin and/or calcium to the reaction because these factors are necessary for enzymatic activity of calcineurin.

In another preferred embodiment of the invention the complex formation is monitored indirectly by analyzing the enzymatic activity of calcineurin. As outlined above the phoshatase activity of calcineurin is strictly dependent on the interaction with superoxide dismutase. Therefore, it is possible to monitor the complex formation indirectly by the measurement of phosphatase activity according to standard procedures. This is especially preferred if the laboratory equipment to perform fluorescent measurements as described above is not available. Furthermore, enzymatic analysis could be used in addition to fluorescence detection like laser fluctuation correlation spectroscopy, e.g. as control.

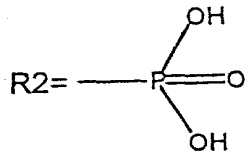
Preferably the enzymatic activity is analyzed by the use of a labeled substrate of calcineurin. The substrate is preferably labeled by fluorescence. One especially preferred substrate is the peptide RII characterized by the sequence:

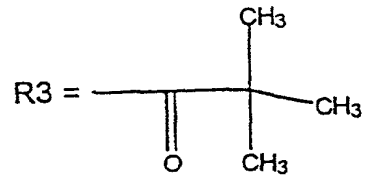
Asp - Leu - Asp - Val - Pro - Ile - Pro - Gly - Arg - Phe - Asp - Arg - Arg - Val - Ser - Val - Ala - Ala - Glu.

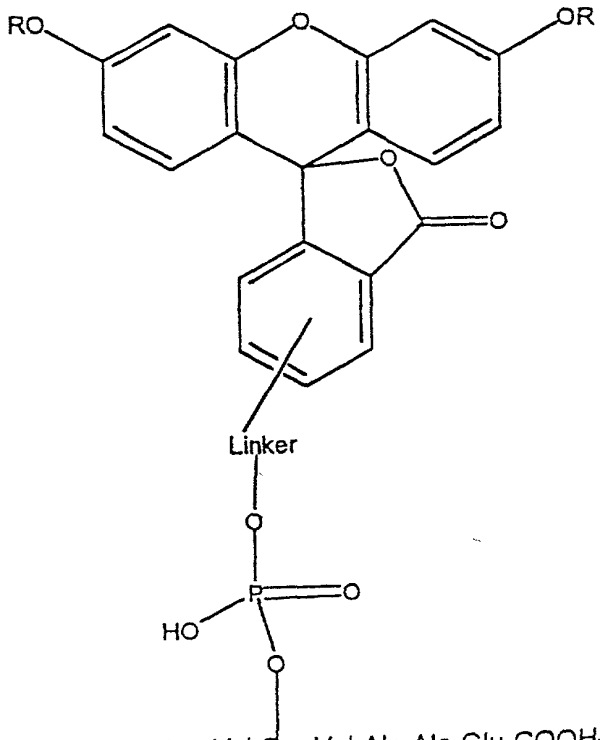
In a preferred embodiment this peptide carries a fluorescent label at serine in position 15. This amino acid is

labeled with fluoresceine by incubating the peptide with fluoresceine-phosphoamidit, thereby providing a labeled substrate (RII-Fluophos). RII interacts with the active center of calcineurin, but it is not converted by the phosphatase. Hereby it is possible to label calcineurin in the active state. Furthermore, it is possible to phosphorylate RII-Fluophos at the fluoresceine moiety as depicted below at tyrosine residues. Due to the phosphorylation RII-Fluophos looses its fluorescence and thereby provides a phosphatase substrate which becomes fluorescent subsequent to dephosphorylation.

R1 = H







-NH2-Asp-Leu-Asp-Val-Pro-Ile-Pro-Gly-Arg-Phe-Asp-Arg-Arg-Val-Ser-Val-Ala-Ala-Glu-COOH-

This peptide could be provided synthetically or it is expressed by cells, especially eucaryotic cells, which have been transfected with appropriate vectors encoding said peptide or other peptides useful as phosphatase substrates. In one embodiment of the invention the fluorescent peptide is used as peptide label in fluorescence microscopy. This provides another method in order to analyze the active state of the calcineurin/superoxide complex.

The inventive method as outlined above is suitable for developing a high-throughput bioassay to identify inhibitors and/or activators of the calcineurin signal pathway. Details are described in the example.

The invention comprises the use of inhibitors of activators of the calcineurin signal pathway for the treatment of acute and/or chronic neurological and cardiovascular diseases like Alzheimer, Parkinson, epilepsy, ischemia and heart-failure. Furthermore the use as immunosuppressive drugs, e.g. in the field of transplantation surgery and inflammatory diseases is included.

Finally the invention comprises a kit for screening of modulators of calcineurin. The kit provides calcineurin and superoxide dismutase enabling complex formation for the screening for modulators of calcineurin as described above. In a first embodiment of the kit the components of the complex are provided as proteins. This kit is suitable for performing the inventive method in vitro. In a second embodiment of the kit the proteins are provided in the form of vectors. These vectors have to be transformed/transfected into cells leading to the expressed proteins. These vectors are prokaryotic or eukaryotic expression vectors, respectively, and could be used to produce the proteins for the in

vitro assay or for the assay using complete cells as described above. In a third embodiment of the inventive kit cells transformed/transfected with the said vectors are provided saving the step of transforming/transfecting for the user. For details of the inventive kit reference is made to the above description.

The new approach to identify new substance classes of calcineurin/CuZnSOD inhibitors comprises inter alia:

- coexpression of CuZnSOD/calcineurin A and calcineurin B to generate a oxidative stable enzyme which is suitable for drug screening,
- efficient purification of CuZnSOD on CuZn-nitrilotriacetat-metal affinity chromatography to retain enzymatic activity,
- efficient purification of calcineurin on Fe-nitrilotriacetat-metal affinity chromatography to retain enzymatic activity and prohibit Fe^{2+} oxidation,
- identifying that mutations in the CuZnSOD associated with a neurological disorder (amyotrophic lateral sclerosis) are also critical for calcineurin-CuZnSOD interaction,
- use of fluorescent labeled recombinant CuZnSOD and calcineurin to screen for CuZnSOD/calcineurin activators or inhibitors,
- use of fluorescent labeled RII-peptide and calcineurin to screen for calcineurin activators or inhibitors,
- identification of calcineurin/CuZnSOD inhibitors or activators by using the recombinant enzymes as affinity ligands to purify new drugs from natural sources,
- inclusion of all isoforms, all known and two newly identified splicevariants into the screening procedure, which allows the identification of less toxic and tissue specific

drugs which are more suitable for the therapeutical treatment of different clinical indications.

The described features of the invention and further features result in greater detail from the examples in combination with the subclaims. The features could be realized in combination with each other or alone.

Example

1. Cloning of CuZnSOD transcript from human brain poly-A-RNA - Cloning of human CuZnSOD was performed by reverse transcription PCR using human brain poly-A-RNA as template (Clontech, Palo Alto, CA, USA). The oligonucleotides SODs1 5'-ttc cgt tgc agt cct cgg aac-3', SODas1 5'-taa ggg gcc tca gac tac atc-3', SOD-PQE60s2 5'-caa gcc atg gcg acg aag gcc gtg tgc gtg ctg-3', SOD-PQE60as2 5'-gaa gat ctt tgg gcg atc cca att aca cca c-3', SOD-PQE30-s2 5'- cgc gga tcc gcg acg aag gcc gtg tgc gtg -3' and SOD-PQE30-as2 5'-ggg ttc gaa tta ttg ggc gat ccc aat tac-3' were supplied by Interactiva (Ulm, Germany). Reverse transcription was performed with the SODas1 primer and 100 ng of poly-A-RNA according to the manufacturer's protocol (Expand reverse transcriptase, Boehringer Mannheim, Germany). The human CuZnSOD cDNA was amplified by nested PCR. The first PCR was performed in 20 µl, using 0,5 µl reverse transcription product, 10 µM SODs1 and SODas1 primers, 300 µM dNTPs, 2 µl of the manufacturer's 10 x PCR buffer and 2.5 U Taq-polymerase with 30 cycles of 1 min 95 ºC, 1 min 45 ºC, 1 min 72ºC followed by a second PCR (50 µl) with 5 µl of the purified first PCR product, 10 µM SOD-PQE60s2 and SOD-PQE60as2 primers, 300 µM dNTPs, 5 µl of the manufacturer's PCR buffer and 2,5 U Taq-polymerase with 30 cycles of 1 min 95 ºC, 1 min 60 ºC, 1 min 72ºC (Taq-polymerase, Pharmacia Biotech, Uppsala, Sweden). For the subcloning

into pQE30 expression vector the primers SOD-PQE30-s2 and SOD-PQE30-as2 were used instead of SOD-PQE60s2/SOD-PQE60as2.

2. Subcloning of human CuZnSOD into pQE60 expression vector (C-terminal fusion protein) - The SOD-pQE60 PCR product was purified by gel extraction prior to NcoI/BglII restriction (New England Biolabs). In order to generate a C-terminal histidin tag fusion protein the CuZnSOD transcript was ligated into the NcoI/BglII treated prokaryotic expression vector pQE60 (QIAexpress expression kit type IV and type ATG, Qiagen, Hilden, Germany). For selection, amplification and sequencing of the CuZnSOD vector construct (CuZnSOD-pQE60), 10 µl ligation product was transformed into E.coli M15[pREP4] cells (QIAexpress expression kit type ATG, Qiagen, Hilden, Germany). Correct reading frames and exclusion of mismatches were confirmed by radioactive and automated sequencing on both strands (T7-sequencing kit, Pharmacia Biotech, Uppsala, Sweden; ABI 377 sequencer, Applied Biosystems, USA).

3. Subcloning of human CuZnSOD into pQE30 expression vector (N-terminal fusion protein) - The SOD-pQE30 PCR product was purified by gel extraction prior to direct ligation into the pCR2.1 vector according to the manufacturer's protocols (TA-Cloning Kit, Invitrogen, De Schelp, Netherlands). After amplification and plasmid purification the pCR2.1-CuZnSOD vector construct was restricted with BamHI to yield a CuZnSOD transcript extended at the 3'-end with the sequence 5'-GAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCC-3' which originates from PCR2.1 vector and includes additional EcoRI/BstX-I/SpeI/BamHI restriction sites. In order to generate a N-terminal histidin tag fusion protein the extended transcript was ligated into the BamHI/HindIII treated prokaryotic expression vector pQE30 (QIAexpress expression kit type IV, Qiagen, Hilden, Germany), blunted by incubation with Klenow-DNA-poly-

merase and circularized by a second treatment with T4-DNA-Ligase (Boehringer Mannheim, Germany). For selection, amplification and sequencing of the CuZnSOD vector construct (CuZn-SOD-pQE30), 10 µl ligation product was transformed into E.coli M15[pREP4] cells (QIAexpress expression kit type IV and type ATG, Qiagen, Hilden, Germany). Correct reading frames and exclusion of mismatches were confirmed by radioactive and automated sequencing on both strands (T7-sequencing kit, Pharmacia Biotech, Uppsala, Sweden; ABI 377 sequencer, Applied Biosystems, USA).

4. Site directed mutagenesis (point mutations associated with the neurological disorder Amyotrophic Lateral Sclerosis and important for calcineurin/CuZnSOD protein interaction) - Amino acid substitutions were introduced according to the manufacturer's protocol, using the primers SOD-PQE60-A4V (5'-caa gcc atg gcg acg aag gtc gtg-3'), SOD-A4V (5'-tcc gcg acg aag gtc gtg tgc gtg ctg-3'), SOD-G37R (5'-gg aag catt aaa aga ctg act gaa ggc-3'), SOD-D90A (5'aat gtg act gct gcc aaa gat ggt gtg-3'), SOD-G93A (5'-gct gac aaa gat gct gtg gcc gat gtg-3'), SOD-AflIII (5'-acg cag gaa aga aca tgt gag caa aag-3'), SOD-BglII (5'-acg cag gaa aga aga tct gag caa aag-3') and the expression vector CuZnSOD constructs CuZn-SOD-pQE30 and CuZnSOD-pQE60, respectively (Chameleon site directed mutagenesis kit, Stratagene, San Diego, CA, USA). Incorporation of the site-directed mutations was confirmed by DNA sequencing of the expression vector. Site directed mutagenesis yielded eight additional vector sequences corresponding to eight protein sequences with clinical relevant amino acid substitutions:

Vector-construct	**amino acid subst. (pos. in protein)**	**nucleic acid subst. (pos. in sequ. prot.)**
CuZnSOD-pQE60	WT = wild-type	= SEQ ID NO 15

CuZnSOD-pQE60-(A4V)	Ala-4 -> Val-4	c-128 -> t-128	
CuZnSOD-pQE60-(G37R)	Gly-37 -> Arg-37	g-226 -> a-226	
CuZnSOD-pQE60-(D90A)	Asp-90 -> Ala-90	a-386 -> c-386	
CuZnSOD-pQE60-(G93A)	Gly-93 -> Ala-93	g-395 -> c-395	

CuZnSOD-pQE30	WT = wild-type = SEQ ID NO 13		
CuZnSOD-pQE30-(A4V)	Ala-4 -> Val-4	c-161 -> t-161	
CuZnSOD-pQE30-(G37R)	Gly-37 -> Arg-37	g-259 -> a-259	
CuZnSOD-pQE30-(D90A)	Asp-90 -> Ala-90	a-419 -> c-419	
CuZnSOD-pQE30-(G93A)	Gly-93 -> Ala-93	g-428 -> c-428	

5. Recombinant expression and purification of wild-type and mutated CuZnSOD - The CuZnSOD-pQE60 or CuZnSOD-pQE30 vector transformed E.coli M15[pREP4] cells were plated on LB / ampicillin (100 µg/ml) / kanamycin (25 µg/ml) agar. Expression cultures were grown in 250 ml LB / ampicillin (100 µg/ml) / kanamycin (25 µg/ml) until the OD_{600} was 0.6. Constitutive leakage expression of human CuZnSOD was fully prevented by the repressor plasmid pREP4-lacI. Production of the human CuZnSOD fusion proteins was induced by addition of IPTG (1 mM). After two hours the bacterial cells were harvested by centrifugation (4000 g, 20 min), resuspended in 8 ml buffer A (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl) and homogenized by three freeze thaw cycles and sonication on ice (Bandelin sonoplus GM70, 300 W, 3 x 10 sec). The lysate was centrifuged (10.000 g, 20 min) and incubated with 750 µl CuZn-NTA (nitrilotriacetat)-agarose for batch affinity binding for 1 h at 4 ºC (Qiagen expressionist kit, Qiagen, Hilden, Germany). CuZn-NTA-agarose was prepared from Ni-NTA-agarose (Qiagen expressionist kit, Qiagen, Hilden, Germany) by subsequent washes in:

1) 2 volumes of bidestilled water
2) 3 volumes of regeneration buffer (6 M guanidiniumhydrochloride, 0.2 M acetic acid)

3) 5 volumes bidestilled water
4) 3 volumes 2% SDS
5) 1 volume 25% ethanol
6) 1 volume 50% ethanol
7) 1 volume 75% ethanol
8) 5 volumes 100% ethanol
9) 1 volume 75% ethanol
10) 1 volume 50% ethanol
11) 1 volume 25% ethanol
12) 1 volume bidestilled water
13) 5 volumes 100 mM Na-EDTA pH 8.0
14) 5 volumes bidestilled water
15) 2 volumes 100 mM $CuSO_4$ / 100 mM $ZnSO_4$ / 1 mM reduced glutathion / 1 mM dithiothreitol
16) 2 volumes bidestilled water
17) 2 volumes regeneration buffer (6 M guanidiniumhydrochloride, 0.2 M acetic acid)
18) 2 volumes buffer buffer A2 (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl, 200 μM $CuSO_4$ / 200 μM $ZnSO_4$ / 1 mM reduced glutathion / 1 mM dithiothreitol

The batch was applied to a 30 ml chromatography column, washed with 15 ml buffer A (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl) and subsequently with 8 ml buffer B (20 mM Tris-HCl pH 7.9, 60 mM imidazole, 500 mM NaCl). C-terminal or N-terminal histidin tagged CuZnSOD was eluted three times with 1,2 ml buffer C (10 mM Tris-HCl, 500 mM imidazole, 250 mM NaCl). Purity and correct expression products were checked by immunoblotting or N-terminal protein sequencing after separation of 20 μl eluate in SDS-PAGE (discontinuous 12,5 % SDS-PAGE). To examine the protein levels in bacterial culture all CuZnSOD variants were induced synchronously at OD_{600} = 0.6 with 1 mM IPTG. After 1 h, 2 h, 3 h, 4 h and 20 h, aliquots (1 ml) of E.coli cultures were

taken, centrifuged and homogenized in buffer A as described. The pellet was resuspended in 1 ml H_2O. Subsequently, 20 µl of the supernatant (soluble fractions) or 20 µl of the sonicated pellet suspension (insoluble fractions) were mixed with 7 µl of denaturing sample buffer (10 % SDS, 10 % mercaptoethanol, 20 % glycerol, 130 mM Tris-HCl pH 6.8, 0.03 % bromphenol blue). The samples were heated for 2 minutes at 80 °C and analyzed by 12 % SDS-PAGE. After coomassie staining, the electropherograms were digitized with a CCD camera (Gel Doc 1000, BioRAD) and analyzed by densitometry using NIH-Image software (1.61).

6. Processing of CuZnSOD - In order to remove the nonphysiological histidin tag and to yield CuZnSOD useful for clinical applications the N-terminal histidin tagged CuZnSOD was proteolytically processed with cathepsin-C or the C-terminal variant was processed with carboxypeptidase-A according to the manufacturer's protocols (Boehringer-Mannheim, Mannheim, Germany). Treatment with cathepsin-C yielded a processed CuZnSOD starting with the amino acids NH_2-GSAT KAVCVLKGDGP (indicated in sequence protocol CuZnSOD-pQE30 SEQ ID NO 13). C-terminal fusion protein was yielded the C-terminal amino acid sequence VIGIAQR-COOH (indicated in sequence protocol CuZnSOD-pQE30 SEQ ID NO 13). Verification was done by peptide sequencing.

7. Reactivation of CuZnSOD - In order to yield physiologically relevant active homodimeric CuZnSOD, the CuZn-NTA eluate was ultrafiltered through a 5 kD membrane (Omegacell, Filtron, Northborough, MA, USA). For buffer exchange the samples were washed three times in reconstitution buffer (50 mM sodium citrate pH 5.5, 1 mM DTT). The protein solutions were incubated at 8 ºC for 7 days (250 µg/ml protein). After distinct time intervals aliquots of the refolding mixture

were either analyzed by native gel electrophoresis (2.6 µg CuZnSOD) and activity staining or assayed in a spectrophotometer (0.5 - 1 µg CuZnSOD, superoxide dismutase assay kit, Calbiochem, San Diego, CA, USA). For visualization of protein bands native gels were stained with coomassie blue. For the production of larger CuZnSOD amounts M15-E.coli cells were subsequently grown in 15 ml, 200 ml, 2500 ml and 20 L flasks. Refolded CuZnSOD proteins were dialyzed against 100 volumes of buffer D (10 mM Tris-HCl 0.1 % Saccharose) and lyophylized.

8. SOD assay and activity staining - Enzymatic activity of the CuZnSOD proteins were either analyzed by 10 % native gel electrophoresis and activity staining with nitrotetrazolium blue dye or by a quantitative spectrophotometrically assay according to published protocols (Beauchamp and Fridovich, 1971; Nebot et al., 1993). Protein yields were determined by the Bradford method (Protein assay kit, BioRAD, Hercules, CA, USA). The concentration of purified CuZnSOD was determined spectrophotometrically using the extinction coefficient $\varepsilon_{265} = 1.84 \times 10^4\ M^{-1}\ cm^{-1}$.

9. Subcloning of human CuZnSOD into pEGFP eukaryotic expression vector and generation of stable transfected PC12 cells (C-terminal fusion protein with enhanced green fluorescent protein as a fluorescent marker/label) - Using 10 µM of the primers SOD-pEGFP-s 5'-ccg cgg gcc cgc cat ggc gac gaa ggc cgt gtg cgt gc-3' and SOD-pEGFP-as 5'-gct cac cat ggt ggt ttg ggc gat ccc aat tac acc ac-3', 10 ng CuZnSOD-pQE60 vector, 300 µM dNTPs, 5 µl of the manufacturer's PCR buffer and 2,5 U Taq-polymerase with 25 cycles of 1 min 95 ºC, 1 min 60 ºC, 1 min 72ºC (50 µl total volume, Taq-polymerase, Pharmacia Biotech, Uppsala, Sweden) a PCR product was generated which was cleaved by ApaI/NcoI digestion. The purified

PCR product was ligated into ApaI/NcoI treated pEGFP-N3 vector (Clontech Laboratories, Palo Alto, CA, USA). After amplification in XL2-Blue cells (25 µg/ ml kanamycin) and plasmid purification the CuZnSOD-pEGFP vector construct was transfected into PC12 rat adrenal pheochromocytoma cells using the CalPhos™ Transfection Kit according to the manufacturer's protocols (Clontech Laboratories, Palo Alto, CA, USA). Stable transfected CuZnSOD-pEGFP clones were selected by fluorescence microscopy (exitation 488 nm/ emission 520 nm, MRC 1024 confocal microscope, BioRAD Laboratories, Hercules, CA, USA).

10. Cloning of the regulatory subunit human calcineurin-B - Cloning of human calcineurin-B was performed by reverse transcription PCR using human brain poly-A-RNA as template (Clontech, Palo Alto, CA, USA). The oligonucleotides CNBa-s1 5'-ccg ccg acc cgc cga gca-3', CNBa-as1 5'-ggt act ctc tga taa gag-3', CNBa-s3 5'-gga att ccc cgg gga aag agg aga aat taa cta tgg gaa atg agg caa gtt atc-3', CNBa-as2 5'-ttc cgg gcc caa gct tct aat taa tca cac atc tac cac cat c-3' were supplied by Interactiva (Ulm, Germany). Reverse transcription was performed with the CNBa-as1 primer and 100 ng of poly-A-RNA according to the manufacturer's protocol (Expand reverse transcriptase, Boehringer Mannheim, Germany). The human calcineurin-B cDNA was amplified by nested PCR. The first PCR was performed in 20 µl, using 0.5 µl reverse transcription product, 10 µM CNBa-s and CNBa-as1 primers, 300 µM dNTPs, 2 µl of the manufacturer's 10 x PCR buffer and 2.5 U Pfu-polymerase with 20 cycles of 1 min 95 ºC, 1 min 55 ºC, 2 min 72 ºC followed by a second PCR (50 µl) with 5 µl of the purified first PCR product, 10 µM CNBa-s3 and CNBa-as2 primers, 300 µM dNTPs, 5 µl of the manufacturer's PCR buffer and 2.5 U Pfu-polymerase with 20 cycles of 1 min 95 ºC, 1 min

55 ºC, 1 min 72ºC (Pfu-polymerase, Stratagene, San Diego, CA, USA).

11. Cloning of the catalytic subunit human calcineurin-A-Alpha and splicevariants - Cloning of human calcineurin-A-alpha was performed by reverse transcription PCR using human brain poly-A-RNA as template (Clontech, Palo Alto, CA, USA). The oligonucleotides CNAa-s1 5'-gcg tcg ctg tcc tcc ggc agc-3', CNAa-as1 5'-gtg aac agg aag tgg tca ctg-3', CNAa-s2 5'-cat gcc atg gatc cat gtc cga gcc caa ggc-3', CNAa-as4 5'-tcc ccc cgg ggta ccc tag tta atc act gaa tat tgc tgc tat tac-3' were supplied by Interactiva (Ulm, Germany). Reverse transcription was performed with the CNAa-as1 primer and 100 ng of poly-A-RNA according to the manufacturer's protocol (Expand reverse transcriptase, Boehringer Mannheim, Germany). The human calcineurin-A-Alpha cDNA was amplified by nested PCR. The first PCR was performed in 25 µl, using 0,5 µl reverse transcription product, 10 µM CNAa-s1 and CNAa-as1 primers, 200 µM dNTPs, 2.5 µl of the manufacturer's 10 x PCR buffer and 1.25 U Pfu-polymerase with 30 cycles of 40 seconds at 95 ºC, 40 seconds at 55 ºC, 3 min 72 ºC followed by a second PCR (25 µl) with 2.5 µl of the purified first PCR product, 10 µM CNAa-s2 and CNAa-as2 primers, 200 µM dNTPs, 2.5 µl of the manufacturer's PCR buffer and 2.5 U Pfu-polymerase with 25 cycles of 40 seconds at 95 ºC, 40 seconds at 55 ºC, 3 min 72 ºC (Pfu-polymerase, Stratagene, San Diego, CA, USA).

Hereby a new splicevariant was identified, which is important for calcium regulation and proteolytic regulation of calcineurin-A. The splicevariant lacks the hole catalytic phosphatase domain and part of calcineurin-binding-site (Elimination of nucleic bases 208-1317 in sequence protocol

CNAa1-pQE30 SEQ ID NO 17). The corresponding vector is named CNAa3-pQE30:

Location/Qualifiers
151..606 /note="splicevariant: Calcineurin A alpha 1 lacking phosphatase domain, newly generated N-terminus exhibits protease activity"
115..150 /note="His-Tag"
649..1161 /note="Calcineurin B;Calcineurin B alpha Ca^{2+} binding"

12. Cloning of the catalytic subunit human calcineurin-A-Beta and splicevariants - PCR was performed as described under 11. with the exception that the primers CNAb-s1 5'-gag cct agc cga gcc ccg gg-3' and CNAb-as1 5'-ctg gga agt agt ggg tca ctg-3' were used for the first PCR and the primers and CNAb-s2 5'- cat gcc atg gat cca tgg ccg ccc cgg agc c-3' and CNAb-as4 5'- tcc ccc cgg ggt acc cta gtt aat cac tgg gca gta tgg ttg cca g-3' were used for second PCR.

13. Cloning of the catalytic subunit human calcineurin-A-Gamma and splicevariants - PCR was performed as described under 11. with the exception that the primers CNAg-s1 5'-gga gcc tgg agg agg ccg ag-3' and CNAg-as1 5'-cgg cag gac tct aag tca tga-3' were used for the first PCR and the primers and CNAg-s2 5'-cat gcc atg gat cca tgt ccg gga ggc gct tc-3' and CNAg-as4 5'-tcc ccc cgg ggt acc cta gtt aat cat gaa tgg gct ttc ttc cct t-3' were used for second PCR.

Hereby a new splicevariant was identified, which is important for calcium regulation and proteolytic regulation of calcineurin-A. The splicevariant with human exon is not yet available in gene database (substitution of nucleic bases 1474-1503 in sequence protocol CNAg2-pQE30 SEQ ID NO 32) with

5'-ACA GTA GAA GCG GTA GAG GCC CGG GAA GCC-3' (corresponding peptide: NH_2-TVEAVEAREA-COOH). The corresponding vector is named CNAg3-pQE30.

Location/Qualifiers
115..150 /note="His-Tag"
151..1689 /note="Calcineurin-A-Gamma-2"
1474..1503 /note="human brain calcineurin-A-gamma alternative exon = interaction domain with cytoskelett, death-domain homolog, stomatin homolog"
1690..1731 /note="RBS&MCS2"
1732..2244 /note="Calcineurin-B"

14. Subcloning of calcineurin-B and calcineurin-A variants into pQE30 - For the recombinant expression in procaryotic cells calcineurin-B was subcloned with either calcineurin-A-alpha1, calcineurin-A-alpha2, calcineurin-A-beta1, calcineurin-A-beta2, calcineurin-A-gamma1 or calcineurin-A-gamma2. The purified calcineurin-A-alpha, calcineurin-B-alpha or calcineurin-A-gamma PCR products (described in 11. - 13.) were restricted with BamHI/XmaI. The purified calcineurin-B product (described in 10.) was restricted with XmaI/HindIII and ligated together with the respective calcineurin-A-fragment into the BamHI/HindIII treated vector pQE30 to yield the final procaryotic expression vector constructs CNAa1-pQE30, CNAa2-pQE30, CNAa3-pQE30, CNAb1-pQE30, CNAb2-pQE30, CNAg1-pQE30, CNAg2-pQE30 and CNAg3-pQE30.

15. Recombinant coexpression and purification of calcineurin-B/calcineurin-A heterodimers with CuZnSOD - CNAa1-pQE30, CNAa2-pQE30, CNAa3-pQE30, CNAb1-pQE30, CNAb2-pQE30, CNAg1-pQE30, CNAg2-pQE30 or CNAg3-pQE30 were transformed into E.coli M15[pREP4][CuZnSOD-pQE30] to yield cells able to coexpress calcineurin-A, calcineurin-B and CuZnSOD. cells

were plated on LB / ampicillin (100 µg/ml) / kanamycin (25 µg/ml) agar. Expression cultures were grown in 250 ml LB / ampicillin (100 µg/ml) / kanamycin (25 µg/ml) until the OD_{600} was 0.6. Constitutive leakage expression was prevented by the repressor plasmid pREP4-lacI. Production of the human calcineurin-A/calcineurin-B histidin tagged heterodimers was induced by addition of IPTG (1 mM). After four hours the bacterial cells were harvested by centrifugation (4000 g, 20 min), resuspended in 8 ml buffer A (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl) and homogenized by three freeze thaw cycles and sonication on ice (Bandelin sonoplus GM70, 300 W, 3 x 10 sec). The lysate was centrifuged (10,000 g, 20 min) and incubated with 750 µl Fe-NTA-agarose for batch affinity binding for 1 h at 4 ºC (Qiagen expressionist kit, Qiagen, Hilden, Germany). Fe-NTA-agarose was prepared from Ni-NTA-agarose (Qiagen expressionist kit, Qiagen, Hilden, Germany) by subsequent washes in:

1) 2 volumes of bidestilled water
2) 3 volumes of regeneration buffer (6 M guanidiniumhydrochloride, 0.2 M acetic acid)
3) 5 volumes bidestilled water
4) 3 volumes 2% SDS
5) 1 volume 25% ethanol
6) 1 volume 50% ethanol
7) 1 volume 75% ethanol
8) 5 volumes 100% ethanol
9) 1 volume 75% ethanol
10) 1 volume 50% ethanol
11) 1 volume 25% ethanol
12) 1 volume bidestilled water
13) 5 volumes 100 mM Na-EDTA pH 8.0
14) 5 volumes bidestilled water
15) 2 volumes 100 mM $FeSO_4$ / 1 mM reduced glutathion / 1 mM dithiothreitol/ 100 mM ascorbic acid

16) 2 volumes bidestilled water
17) 2 volumes regeneration buffer (6 M guanidiniumhydrochloride, 0.2 M acetic acid)
18) 2 volumes buffer buffer A3 (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl, 200 μM $FeSO_4$ / 1 mM reduced glutathion / 1 mM dithiothreitol/ 1 mM ascorbic acid

The batch was applied to a 30 ml chromatography column, washed with 15 ml buffer A4 (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl/ 1 mM reduced glutathion / 1 mM dithiothreitol/ 1 mM ascorbic acid) and subsequently with 8 ml buffer B (20 mM Tris-HCl pH 7.9, 60 mM imidazole, 500 mM NaCl/ 1 mM reduced glutathion / 1 mM dithiothreitol/ 1 mM ascorbic acid). N-terminal histidin tagged calcineurin-A/calcineurin-B heterodimer was eluted three times with 1.2 ml buffer C (10 mM Tris-HCl, 500 mM imidazole, 250 mM NaCl/1 mM reduced glutathion / 1 mM dithiothreitol/ 1 mM ascorbic acid, buffer was degased and subsequently saturated with nitrogen). To prevent oxidation of calcineurin, the eluate was stored at -80° ºC in nitrogen containing and oxygen free vials. Purity and correct expression products were checked by immunoblotting or N-terminal protein sequencing after separation of 20 μl eluate in SDS-PAGE (discontinuous 12,5 % SDS-PAGE).

16. Subcloning of human calcineurin-A-Alpha into pEGFP eukaryotic expression vector and generation of stable transfected PC12 cells (C-terminal fusion protein with enhanced green fluorescent protein as a fluorescent marker) - The vector CNAa2-pQE30 was digested with BamHI/XmaI to generate a sticky end CNAa2 fragment. The purified fragment was ligated into Bgl-II/XmaI treated pEGFP-C1 vector (Clontech Laboratories, Palo Alto, CA, USA). After amplification in XL2-Blue cells (25 μg/ ml kanamycin) and plasmid purification the

CNAa-pEGFP vector construct was transfected into PC12 rat adrenal pheochromocytoma cells using the CalPhos™ Transfection Kit according to the manufacturer's protocols (Clontech Laboratories, Palo Alto, CA, USA). Stable transfected CNAa-pEGFP clones were selected by fluorescence microscopy during a three month propagation procedure (exitation 488 nm/emission 520 nm, MRC 1024 confocal microscope, BioRAD Laboratories, Hercules, CA, USA).

17. Subcloning of calcineurin-A-Beta into pEGFP - The same procedure as described in 16. was applied except that the CNAa2-pQE30 vector was substituted by CNAb2-pQE30 to generate CNAb-pEGFP.

18. Subcloning of calcineurin-A-Gamma into pEGFP - The same procedure as described in 16. was applied except that the CNAa2-pQE30 vector was substituted by CNAg2-pQE30 to generate CNAg-pEGFP.

19. Western blotting and protein sequencing - Transfer of purified proteins from 12% SDS-PAGE to PVDF membranes (Boehringer-Mannheim, Mannheim, Germany) was performed according to standard protocols using transfer buffer (48 mM Tris, 39mM Glycine, 20% methanol, 1% SDS, pH 9.2) and following blotting conditions: 75 min at 25 V/110 mA. Blocking, washing and detection (HRP detection system) were performed according to the manufacturer's protocols (ECL kit, Amersham, Buckinghamshire, UK). An anti-human CuZnSOD antibody (1:5,000 dilution, rabbit polyclonal anti-human SOD1 antibody; BIOMOL, Hamburg, Germany) was used as primary antibody and an anti-rabbit IgG antibody (1:10,000 dilution) labeled with HRP was used as secondary antibody. For the detection of calcineurin-A (alpha, beta, gamma isoforms) a polyclonal calcineurin-A antibody was used as 1:5000 dilution (Sigma Aldrich, Deisen-

hofen, Germany). For N-terminal protein sequencing the PVDF membrane was soaked in 100% methanol. Proteins which seemed to be blocked by N-terminal posttranslational modifications were treated with acylamino-acid-peptidase according to the manufacturer's protocol (Boehringer-Mannheim, Mannheim, Germany). Coomassie brilliant blue stained bands were cut out. Automated Edman degradation of peptides was performed on an Applied Biosystems protein sequencer (476A).

20. Calcineurin phosphatase assay - 100 ng - 4 µg recombinant calcineurin (calcineurin-A/B heterodimer), 100 ng - 1 µg purified bovine brain calcineurin (Sigma Aldrich, Deisenhofen, Germany) or 100 µg homogenized tissue or cell extracts were used for classical calcineurin phosphatase assays. 100 µg cells or tissue were homogenized exactly as described (Stemmer et al., 1995). Partly purified and redox sensitive calcineurin was prepared by centrifugation at 14,000 rpm at 4°C for 10 min (Eppendorf Centrifuge 5417R) and the resulting supernatant was separated on a 1.5 x 10 cm Sephadex-G50 gelfiltration column as described (Stemmer et al., 1995), (Gold et al., 1997). Phosphotyrosine phosphatase assay was performed in microplates (100 µl total assay volume) either using 30 µM fluoresceinmonophosphate or 20 mM para-nitrophenylphosphate (Sigma Aldrich, Deisenhofen, Germany); 10 µl recombinant, purified or partly purified and assay buffer (25 mM Tris/HCl, pH 7; 2 mM $CaCl_2$; 0.1 µM calmodulin; 25 µM FK506). After starting the enzymatic reaction with para-nitrophenylphosphate or fluoresceinmonophosphate the absorbance at 405 nm (para-nitrophenylphosphate) or fluorescence (λ exication = 485 nm; λ emission = 520 nm) was monitored over 20 min at 30°C using a UV/VIS/fluorescence microplate photometer (Biolumin 960 kinetic fluorescence/absorbance photometer, Molecular Dynamics). Phosphoserine phosphatase assay was performed as described (Hubbard and Klee, 1991), (Wang et

al., 1996). In short: 40 µl recombinant or partly purified calcineurin was mixed with test buffer (40 mM Tris/HCl pH 8; 0.1 M KCl; 0.4 mg/ml BSA; 0.67 mM DTT; 0.67 µM calmodulin; 1 µM FKBP binding protein; 0.5 µM ocadaic acid for inhibition of phophatase A1 and A2) and enzymatic reaction and calcium induced redox-inactivation of calcineurin started by addition of 20 µl substrate buffer (7.7 µM radioactive phosphorylated RII-peptid, 2.0 mM $CaCl_2$). The assay was performed in duplicates and the addition of 1 µM FK506 or cyclosporine was used to verify calcineurin activity for each reading point. The protective effect of CuZnSOD against redox inactivation of calcineurin was determined by addition of 3 µg recombinant human wild-type or mutated CuZnSOD (constant CuZnSOD protein) or addition of 1.67 units of recombinant human wild-type or mutated CuZnSOD (constant CuZnSOD activity). The reaction mixture was incubated for 2 min at 30°C and stopped with 100 mM potassium phosphate / 5% TCA. The reaction mixture was passed through a 0.5 ml ion-exchange column (Dowex; AG 50W-X8, BioRad) and the unbound phosphate eluted with 0.5 ml water. The quantity of released phosphate was determined by a scintillation counting.

An enzymatic protein phosphatase assay was established using the nonphysiological substrate fluoresceinmonophosphate (FMP). Assuming a Michaelis-Menten kinetic for FMP and using the Lineweaver-Burk method for analysis of kinetic data a K_M of 40 µM and a V_{max} of 400 µmol / min was determined. The assay was applicable to calcineurin and magnesium dependent proteinphosphatase 2C (data not shown, (Grothe et al., 1998)). The enzymatic activity is linear in the range of 12.5 pM to 75 pM calcineurin. FMP is more sensitive than para-nitrophenylphosphate (pNPP). Neither FMP nor pNPP are useful to measure calcineurin activity in crude preparations by inhibition with the immunsuppressive drugs FK506 or cyclosporine

(cell homogenate, partly purified calcineurin). Both substrates also failed to measure calcium induced redox-inactivation of calcineurin or CuZnSOD mediated protection of this inactivation. The inhibition assay also failed when calcium was substituted against other divalent cations (Ni^{2+}, Mg^{2+}). Only the physiological relevant substrate could be used in an immunsuppressive drug inhibitory assay (RII-peptide phosphopeptide). In the classic radioactive assay 95% inhibition with 1 μM FK506 or cyclosporine was determined. It is concluded that inhibition of calcineurin activity by immunosuppresive drugs needs larger molecular weight substrates than pNPP and FMP. Furthermore it is concluded that redoxsensitivity is linked to phosphoserine phosphatase activity and therefore not detectable with phosphotyrosine analoges like pNPP or FMP. The recombinant human wild-type CuZnSOD and purified human erythrocyte CuZnSOD (Sigma Aldrich, Deisenhofen, Germany) were effective to protect 50-100% of calcineurin after calcium induced redox inactivation. Mutated CuZnSOD proteins, associated with the severe neurological disorder amyotrophic lateral sclerosis, were less effective to protect calcineurin against redox inactivation.

Protective effect of CuZnSOD of calcium induced inactivation of calcineurin

	Percentage of FK506 inhibitable RII-phosphopeptide activity after 20 min compared with the activity at 0 min	
human CuZnSOD	**constant protein (3 μg)**	**constant activity (1.67 U)**
erythrocyte wild-type (8330 U/mg)	57 +/-10 %	57 +/-10 %
recombinant wild-type	70 +/-33 %	58 +/-22 %

(6380 U / mg)		
recomb. mutation D90A (4590 U/ mg)	42 +/-17 %	32 +/-15 %
recomb. mutation G93A (2130 U/ mg)	16 +/-16 %	21 +/-22 %
recomb. mutation A4V (1820 U/ mg)	22 +/-27 %	8 +/-3 %
control (no CuZnSOD) (0 U / mg)	9 +/-7 %	9 +/-7 %

The protective effect does not depend on CuZnSOD activity since higher protein amounts of mutated CuZnSOD corresponding to a higher enzymatic activity were even less effective in protection of calcineurin.

Therefore it is concluded that amino acid substitutions, associated with familial amyotrophic lateral sclerosis, are important for the protein interaction of calcineurin and CuZnSOD and therefore are involved in the CuZnSOD mediated protection of calcium induced redox inactivation of calcineurin. Since this protective effect is disturbed in amyotrophic lateral sclerosis and protection of calcineurin by CuZnSOD it may also be important in other neurological and cardiovascular diseases (Alzheimer, Parkinson, epilepsy, ischemia, heart-failure).

An high-throughput bioassay was developed to detect and isolate artificial or endogenous drugs enhancing (activators) CuZnSOD-calcineurin interaction and therefore protecting calcineurin against redox-inactivation or drugs reducing (inhibitors) CuZnSOD-calcineurin interaction and therefore inhibit calcineurin activity. Inhibitors are useful to substitute toxic immunsuppressive drugs like FK506 or cyclosporine. Activators and inhibitors may be useful for the

therapeutical treatment of amyotrophic lateral sclerosis, Parkinson, Alzheimer, epilepsy, ischemia and cardiovascular diseases.

21. High Throughput BioAssay using recombinant calcineurin-A, recombinant calcineurin-B, calmodulin and recombinant CuZnSOD (analytical assay to identify activators or inhibitors of CuZnSOD/calcineurin interaction) - Laser fluctuation correlation spectroscopy (FCS) is a useful tool to quantify ligand-ligand interactions. The fluorescence F(t) of a optical well defined volume element which is excited by a confocal laser is monitored as a function of time. The temporal autocorrelation of the fluorescence fluctuation δ F(t) yields the time scale of this dynamics and the average number of independent fluorophores in the probe volume. If the fluorescene fluctuation arise from diffusive motion and from fluorescence sensitive reaction, fluorescent fluctuation correlation function signal is approximated by the formula:

$$G_{DR}(\tilde{\tau}) = G_{Diff}(\tilde{\tau}) * [1+A * \exp(-k_R * \tilde{\tau})]$$

$\tilde{\tau}$ = fluorescence correlation time

k_R = apparent binding constant of the fluorescent labeled ligand

A = equilibrium coefficent dependent constant

If one measurement is performed with a solution only containing the fluorescent labeled ligand and a second measurement is performed with a solution containing the fluorescent labeled ligand and an interacting molecule the correlation function G_{DR} can be separately analyzed and yields binding parameters of the interacting molecules. Upon binding of the ligand to the interacting molecule the hydrodynamic radius increases and therefore the diffusion coefficient decreases resulting in a longer correlation time.

A fluorescence labeled recombinant CuZnSOD as a fluorescent label was used to monitor the binding dynamics to calcineurin. CuZnSOD was labeled with Oregon-Green-514 dye according to the manufacturer's protocols (FluoReporter Protein labeling Kit, Molecular Probes, Leiden, Netherlands). The amount of fluorescent dye labels per CuZnSOD dimer was quantified by determining the ratio of the absorbance at 265 nm (CuZnSOD protein) / 514 nm (Oregon-dye). The diffusion constant and correlation time of the labeled CuZnSOD (100 nM) was measured on a bovine serum albumin treated glass plate with an confocal laser microscope attached to an autocorrelator (λ excitation = 488 nm, λ emission = 511 nm) in 10 µl assay buffer containing 50 mM sodiumphosphate pH 7.1, 150 mM NaCl, 0.67 mM DTT, 0.67 µM calmodulin, 0.67 mM $CaCl_2$, 1 mM $MgCl_2$. The beam from a modelocked Ti:Sa or cw argon ionlaser was collimated to fill the back aperture of a immersion microscope objective (Zeiss C-Apochromat 63x1.2w), producing a small diffractionlimited spot. The emitting fluorescent light was collected by the same objective separated from the excitation light by a beamsplitter/filter combination and imaged first to a variable pinhole and than to the detector (Avalanche Photodiode EG&G SPCM AQ161 or PMT Hamamatsu R5600-03). The labeled CuZnSOD exhibited an autocorrelation time corresponding to a hydrodynamic radius of 41,000 Dalton which is comparable to the expected molecular weight of the homodimer (34,600 Dalton). Next 0.2 µl calcineurin-A/B heterodimer (5 µM) was added to a labeled CuZnSOD mixture and the fluorescence correlation signal was determined. The hydrodynamic radius increases from 41 kDa to 90 kDa indicating that approximatly one calcineurin heterodimer interacts with one CuZnSOD dimer (expected: 114 kDa). Using mutated D90A CuZnSOD yielded an apparent molecular weight of 180.000 kDa indicating the formation of calcineurin/CuZnSOD aggrega-

tes. The apparent binding constant between human wild-type CuZnSOD and calcineurin was estimated as k_D = 2 x 10^{-6} M +/- 1 x 10^{-6} M. It is concluded that laser correlation spectroscopy is useful to perform a ultra high throughput screening for ligands diminishing the CuZnSOD/calcineurin interaction which simply can be monitored by a reduction of the autocorrelation time after addition of a potential drugs. It is possible to screen for suitable substances using substances available in chemical, peptide or natural compound screening libraries.

22. High Throughput BioAssay using recombinant calcineurin-A, recombinant calcineurin-B, calmodulin and RII-Fluophos (analytical assay to identify activators or inhibitors of calcineurin) - RII peptide was synthesized according to standard peptide synthesis protocols ((Blumenthal et al., 1988); Interactiva, Ulm, Germany). To generate a fluorescent labeled peptide which furthermore contains a phosphoester at Ser-15, amino acid residue Ser-15 was coupled with fluoresceine-phosphoamidit (FluoreDite Labeling Reagent, Perseptive Biosystems), which is usually used for labeling of nucleotides, to yield RII-Fluophos (Interactiva, Ulm, Germany). The expected molecular weight (2578.8 Dalton) was confirmed by mass spectrometry (2580.6 Dalton). The Fluophos-RII-peptide was not converted by calcineurin as was monitored by fluorescence spectrometrie (Biolumin 960 UV-/VIS/fluorescence microplate reader). Therefore Fluophos-RII-peptide was used in laser fluorescence correlation spectroscopy as described in paragraph 20 exept that λ excitation was 488 nm and λ emission was 520 nm. Furthermore, labeled CuZnSOD was substituted by 10 nM Fluophos-RII-peptide yielding a hydrodynamic radius corresponding to 4 kDa (expected 2.6 kDa). After calcineurin addition the molecular weight increases to 100.000 kDa and a binding

constant of $K_d = 0.6 \times 10^{-6}$ M is estimated. Binding constants were comparable between the six calcineurin isoforms/splicevariants. It is concluded that laser correlation spectroscopy is useful to perform a ultra high throughput screening for ligands directly substrate binding to calcineurin by simply monitoring the autocorrelation time after addition of potential drugs. By descriminating the binding properties of potential drugs to the six different heterodimer combinations (calcineurin-A-alpha1/calcineurin-B, calcineurin-A-alpha2/calcineurin-B, calcineurin-A-beta1/calcineurin-B, calcineurin-A-beta2/calcineurin-B, calcineurin-A-gamma1/calcineurin-B, calcineurin-A-gamma2/calcineurin-B) it is possible to identify tissue specific and therefore less toxic calcineurin inhibitors.

It is possible to combine the screening procedures described in paragraph 20 and 21 strategically: substances which are able to inhibit the calcineurin-CuZnSOD interaction (positive hit in paragraph 20) but failed to show an effect in procedure 21 (negative hit) are predominantly positive candidates for the therapeutical use in neurological disorders because a toxic immunosuppressive side effect is less probable. Substances which fail to inhibit the calcineurin-CuZnSOD interaction (negative hit) but show an effect in procedure 21 (positive hit) are predominantly positive candidates for immunosuppression. Substances effective in both procedures are likely to be toxic.

23. Cellular BioAssay using eucaryotic cells transfected with calcineurin-A-EGFP fusionprotein or CuZnSOD-EGFP fusion protein - PC12 cells stably transfected with CuZnSOD and calcineurin isoenzymes serve as a model for monitoring the effects of CuZnSOD or calcineurin overexpression in neuronal cells. CuZnSOD reportedly has been implicated to be involved

in the mediation of hypoxie tolerance, whereas calcineurin overexpression is associated with epileptogenesis, Parkinsonism or Alzheimers disease. It is possible to use theses cells subsequently to the identification of potential drugs in screening protocols 20 und 21. Toxicity of potential neuroprotective drugs and the effect on the subcellular distribution of calcineurin-isoforms or CuZnSOD, respectively can be monitored.

24. Pull-Down-BioAssay using histidine tagged recombinant CuZnSOD to purify CuZnSOD interacting ligands (Preparative assay to isolate activators or inhibitors of CuZnSOD/calcineurin interaction from biological sources) - Recombinant purified histidine tagged CuZnSOD in 50 mM sodiumphosphate buffer pH 8.0 was attached to CuZn-NTA magnetic agarose beads by incubating 100 µl beads suspension with 100 µl CuZnSOD solution (0.3 µg/µl) in 96 well microplates for 30 minutes at room temperature on a microplate shaker (600 rpm). CuZn-NTA magnetic beads were generated from Ni-NTA beads by applying the same procedure as described under paragraph 5 (Ni-NTA magnetic agarose beads, Qiagen, Hilden, Germany). The microplate was placed on the 96 well magnet for 1 minute and the supernatant removed from the wells.

Cytosolic ligands were isolated as follows: 200 µl interaction buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole pH 8.0, 0.1 % Tween-80) were added to the CuZn-NTA agarose beads/CuZnSOD containig wells and placed on the 96 well magnet to remove interaction buffer. 100 mg tissue, cells or other biological specimen to be analyzed for CuZnSOD interacting ligands were homogenized in 200 µl lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 8.0, 0.1 % Tween-80) using a dounce homogenizer. The lysate was cleared by 30 min centrifugation at 10,000 g at 4 ºC. The supernatant was

applied to the wells containing CuZn-NTA absorbed recombinant human CuZnSOD, mixed and incubated for 60 minutes at 0 ºC. The microplate was placed on the 96 well magnet for 1 minute to remove the supernatant. After removal of the lysate the wells were washed twice by adding 200 µl interaction buffer. Elution of CuZnSOD and interacting ligands was achieved by addition of 100 µl elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole pH 8.0, 0.1 % Tween-80).

Membranous ligands were isolated as follows: denaturing interaction buffer (6 M guanidine-HCL, 100 mM NaH_2PO_4 pH 8.0, 0.1 % Tween-80) was added to the CuZn-NTA agarose beads/ CuZnSOD containig wells and placed on the 96 well magnet to remove interaction buffer. The pellet from the procedure above was solubilized in 200 µl denaturing interaction buffer (6 M guanidine-HCL, 100 mM NaH_2PO_4 pH 8.0, 0.1 % Tween-80) for 60 minutes at room temperature. The solubilisate was cleared by 30 min centrifugation at 10,000 g at room temperature. The supernatant was applied to the wells containing CuZn-NTA absorbed recombinant human CuZnSOD, mixed and incubated for 60 minutes at room temperature. The microplate was placed on the 96 well magnet for 1 minute to remove the supernatant. The wells were washed once with 200 µl denaturing interaction buffer (6 M guanidine-HCL, 100 mM NaH_2PO_4 pH 8.0, 0.1 % Tween-80) and a second time with 200 µl denaturing wash buffer (8 M Urea, 100 mM NaH2PO4 pH 8.0, 0.1 % Tween--80). Elution of CuZnSOD and interacting ligands was achieved by addition of 100 µl denaturing elution buffer (8 M Urea, 100 mM NaH_2PO_4 pH 4.0, 0.1 % Tween-80).

To remove low molecular weight ligands for HPLC analysis, the eluates (cytosolic or membranous) were ultrafiltered through a 5 kDa membrane as described under 7. Low molecular weight ligands were separated on a preparative reverse phase HPLC

(UV detection at 200 nm). Homogeneity and molecular weight of UV detectable fractions were analyzed by mass spectrometry. High molecular weight ligands (ultrafiltration remainder) were separated on a 10% polyacrylamide gel and protein bands identified by sequencing or MALDI mass spectrometrie as described under 19. Interacting nucleic acid was analyzed by separating the membranous eluate on a 1 % agarose gel and staining with ethidium bromide. Fluorescent bands were extracted from the agarose (Qiagen gel extraktion kit, Qiagen, Hilden, Germany) subjected to digestion with RsaI and subcloned into RsaI treated pQE30 vector for DNA sequencing.

25. Pull-Down-BioAssay using histidine tagged recombinant calcineurin-A and calcineurin-B to purify calcineurin interacting ligands (Preparative assay to isolate activators or inhibitors of CuZnSOD/calcineurin interaction from biological sources) - Isolation and identification of calcineurin interacting ligands was performed analogous to paragraph 24 with the exception that recombinant calcineurin-A/B heterodimer was attached to Fe-NTA magnetic agarose beads which were prepared as described under paragraph 15. Furthermore six different heterodimer combinations were used (calcineurin-A-alpha1/calcineurin-B, calcineurin-A-alpha2/calcineurin-B, calcineurin-A-beta1/calcineurin-B, calcineurin-A-beta2/calcineurin-B, calcineurin-A-gamma1/calcineurin-B, calcineurin-A-gamma2/calcineurin-B) to descriminate between isoenzyme and splicevariant specific interaction partners.

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Content of sequence listing

1. eukaryotic expression vector CuZnSOD-EGFP (CuZnSOD-pEGFP) (DNA)
2. CuZnSOD (PRT)
3. EGFP (PRT)
4. eukaryotic expression vector EGFP-Calcineurin A alpha (CNAa-pEGFP) (DNA)
5. EGFP (PRT)
6. Calcineurin A alpha (PRT)
7. eukaryotic expression vector EGFP-Calcineurin A beta (CNAb-pEGFP) (DNA)
8. EGFP (PRT)
9. Calcineurin A beta (PRT)
10. eukaryotic expression vector EGFP-Calcineurin A gamma (CNAg-pEGFP) (DNA)
11. EGFP (PRT)
12. Calcineurin A gamma (PRT)

13. prokaryotic expression vector His-CuZnSOD (CuZnSOD-pQE30) (DNA)
14. CuZnSOD (PRT)
15. prokaryotic expression vector CuZnSOD-His (CuZnSOD-pQE60) (DNA)
16. CuZnSOD (PRT)
17. prokaryotic expression vector His-Calcineurin A alpha1-Calcineurin B (CNAa1-pQE30) (DNA)
18. Calcineurin A alpha1 (PRT)
19. Calcineurin B (PRT)
20. prokaryotic expression vector His-Calcineurin A alpha2-Calcineurin B (CNAa2-pQE30) (DNA)
21. Calcineurin A alpha2 (PRT)
22. Calcineurin B (PRT)
23. prokaryotic expression vector His-Calcineurin A beta1-Calcineurin B (CNAb1-pQE30) (DNA)
24. Calcineurin A beta1 (PRT)
25. Calcineurin B (PRT)
26. prokaryotic expression vector His-Calcineurin A beta2-Calcineurin B (CNAb2-pQE30) (DNA)
27. Calcineurin A beta2 (PRT)
28. Calcineurin B (PRT)
29. prokaryotic expression vector His-Calcineurin A gamma1-Calcineurin B (CNAg1-pQE30) (DNA)
30. Calcineurin A gamma1 (PRT)
31. Calcineurin B (PRT)
32. prokaryotic expression vector His-Calcineurin A gamma2-Calcineurin B (CNAg2-pQE30) (DNA)
33. Calcineurin A gamma2 (PRT)
34. Calcineurin B (PRT)
35. peptide RII (PRT)

Amended claims

Claims:

1. Method for screening of modulators of calcineurin activity, characterized in that an interaction between calcineurin and superoxide dismutase is monitored, comprising the following steps
   - forming of a complex comprising at least calcineurin and superoxide dismutase under incubation with at least one potential modulator,
   - detecting the influence of the potential modulator by directly monitoring the complex formation and/or by monitoring the activity, especially the enzymatical activity of the complex.

2. Method according to claim 1, characterized in that the superoxide dismutase is a Copper/Zinc-superoxide dismutase.

3. Method according to claim 1 or 2, characterized in that forming of the complex is performed in the presence of the potential modulator.

4. Method according to claim 1 or 2, characterized in that the potential modulator is added after the complex has been formed.

5. Method according to one of the preceding claims, characterized in that the monitoring is performed by detection of labels, especially fluorescent labels.

6. Method according to one of the preceding claims, characterized in that calcineurin and/or superoxide dismutase

carry labels, especially fluorescent markers, wherein preferably the labels are enhanced green fluorescent protein.

7. Method according to claim 6, characterized in that calcineurin and/or superoxide dismutase are expressed as fluorescent proteins, particularly as fusion proteins together with enhanced green fluorescent protein.

8. Method according to one of the preceding claims, characterized in that the monitoring of complex formation is performed by laser fluctuation correlation spectroscopy.

9. Method according to one of the preceding claims, characterized in that calcineurin and superoxide dismutase are coexpressed in cells, especially in eukaryotic cells, and that the complex formation is performed within the cell.

10. Method according to one of the preceding claims, characterized in that calcineurin and/or superoxide dismutase are expressed in cells, especially in prokaryotic cells, and that calcineurin and/or superoxide dismutase are isolated and/or purified before the complex formation is performed.

11. Method according to claim 10, characterized in that purification of calcineurin is achieved by affinity chromatography, especially by ferro-nitrilotriacetat-metal affinity chromatography.

12. Method according to claim 10, characterized in that purification of superoxide dismutase is achieved by

affinity chromatography, especially by copper/zinc-nitrilotriacetat-metal affinity chromatography.

13. Method according to one of the preceding claims, characterized in that in the complex formation step additionally calmodulin and/or calcium is added.

14. Method according to one of the preceding claims, characterized in that the monitoring of the enzymatical activity is performed by analyzing the phosphatase activity of calcineurin.

15. Method according to claim 14, characterized in that the phosphatase activity is analyzed by the use of at least one substrate, which preferably carries a label, especially a fluorescent label.

16. Method according to claim 15, characterized in that the substrate is a peptide, especially a peptide characterized by the amino acid sequence

Asp - Leu - Asp - Val - Pro - Ile - Pro - Gly - Arg - Phe - Asp - Arg - Arg - Val - Ser - Val - Ala - Ala - Glu.

17. Method according to claim 15 or 16, characterized in that the substrate is a peptide containing a residue, especially a serine residue, labeled with fluoresceine.

18. Method according to one of claims 3 to 17, characterized in that prior or after detecting the influence of the potential modulator on the complex formation and/or complex activity the influence of the potential modulator on the activity, especially the enzymatical activity of calcineurin is detected seperately.

19. Method for screening of modulators of calcineurin activity, especially according to one of the preceding claims, comprising

a) determining the interaction of a potential modulator with either calcineurin or superoxide dismutase as a partner,

b) taking a potential modulator showing interaction with calcineurin or superoxide dismutase according to step a),

c) determining the interaction of said modulator taken in step b), with the other partner, namely calcineurin or superoxide dismutase, respectively, and

d) identifying the potential modulator showing interaction also according to step c).

20. Method according to claim 19, characterized in that calcineurin and/or superoxide dismutase comprises at least one tag, especially a histidine tag.

21. Method according to claim 19 or claim 20, characterized in that said superoxide dismutase is a Copper/Zinc-superoxide dismutase.

22. Method according to one of claims 19 to 21, characterized in that calcineurin and/or superoxide dismutase is attached to a solid matrix, especially a Ni-NTA, Fe-NTA and/or CuZn-NTA matrix.

23. Kit for screening of modulators of calcineurin activity comprising

- calcineurin and/or a vector encoding for calci-

neurin and/or cells capable of expressing calcineurin, and

- superoxide dismutase and/or a vector encoding for superoxide dismutase and/or cells capable of expressing superoxide dismutase.

- - - - -

A 35 594 PCT/US

#4

DECLARATION FOR PATENT APPLICATION	Attorney Docket 24498

As a below-named inventor(s), I/we hereby declare that

My/Our residence(s), post office address(es) and citizenship(s) is/are as stated below next to my/our name(s)

I/We believe I/we am/are the original inventor, first and sole (*if only one name is listed below*) or the original, first and joint inventors (*if plural names are listed below*) of the subject matter which is claimed, and for which a patent is sought on the invention entitled:

Method for screening of modulators of calcineurin activity

the specification of which: (check one)

[ ] is attached hereto.

[X] was filed on 22 July 1999, as Serial No. PCT/EP99/05220,
19 Jan. 2001 09/744,016
and was amended on _____ 19 _____ (if applicable).

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of this application as defined by 37 CFR § 1.56.

We hereby claim foreign priority benefits under 35 U.S.C. § 119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Applications:

(Application No.)	(Country)	(Day/Month/Year Filed)	Priority Claimed [X] Yes	[ ] No
98 11 3876.1	~~xxxxxx~~ Europe	22 / 07 / 1998	[X]	[ ]
_____	_____	/ /	[ ]	[ ]
_____	_____	/ /	[ ]	[ ]

We hereby appoint Gary M. Nath, Reg. No. 26,965; Harold L. Novick, Reg. No. 26,011; Suet M. Chong, Reg. No. 38,104; Todd L. Juneau, Reg. No. 40,669; Patricia M. Drost, Reg. No. 29,790; Lee C. Herman, Reg. No. 41,827; Jerald L. Meyer, Reg. No. 41,194; Joshua B. Goldberg, Reg. No. 44,126; David Milligan, Reg. No. 42,893 and Robert G. Lev, Reg. No. 30,280; David R. Murphy, Reg. No. 22,751; Paul A. Sacher, Reg. No. 43,418; Gregory B. Kang, Reg. No. P-45,273; Scott F. Yarnell, P-45,245; as my attorneys to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith.

Direct Telephone Calls to:

Gary M. Nath
(202)775-8383

Send Correspondence to:

Nath & Associates
Sixth Floor, 1030 15th Street,
N.W., Washington, D.C. 20005 - USA

We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by 35 U.S.C. § 112, first paragraph, I/we acknowledge the duty to disclose material information as defined in 37 CFR § 1 56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(U.S. Application Serial No.)	(U.S Filing Date)	(Status--patented, pending, abandoned)
_____	_____	_____
_____	_____	_____

DECLARATION FOR PATENT APPLICATION	Attorney Docket: 24498

We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: Helge VOELKEL

Inventor's Signature H. Völkl Date 21/02/2001

Residence: D-98081 Ulm, Germany DEX

Country of Citizenship: Loherstrasse 18/1, D-98081 Ulm, Germany

Post Office Address: _____

Full name of second inventor: _____

Inventor's Signature _____ Date _____

Residence: _____

Country of Citizenship: _____

Post Office Address: _____

Full name of third inventor: _____

Inventor's Signature _____ Date _____

Residence: _____

Country of Citizenship: _____

Post Office Address: _____

Full name of fourth inventor: _____

Inventor's Signature _____ Date _____

Residence: _____

Country of Citizenship: _____

Post Office Address: _____

A 35 594 PCT/US

#4

Applicant/Patentee: Helge VOELKEL
Serial/Patent No.: 09/744,016 Atty. Dkt No. 24498
Filed on/Issued on:
For: Method for screening of modulaters of calcineurnin activity

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR § 1.9(f) AND § 1.27(b)) - SMALL BUSINESS CONCERN

I hereby declare that I am:
[ ] The owner of the small business concern identified below:
[ ] An official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Bioinfact GmbH
ADDRESS OF CONCERN Loherstrasse 18, DE - 89081 Ulm, Germany

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR § 121.8-18, and reproduced in 37 CFR § 1.9(d), for purposes of paying reduced fees under 35 USC § 41(a) and (b), in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement: (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that the rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled Method for screening of modulators of calcineurin by inventor(s) Helge VOELKEL described in: activity

[X] The specification filed herewith
[ ] U.S. Application Serial No. _____, filed _____
[ ] U.S. Patent No. _____, issued _____

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR § 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR § 1.9(d) or a non-profit organization under 37 CFR § 1.9(e).

FULL NAME _____

ADDRESS _____

[ ] Individual [ ] Small Business [ ] Non-Profit

*NOTE: Separate Verified Statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR § 1.27).

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the Issue Fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC § 1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this Verified Statement is directed.

NAME OF PERSON SIGNING Dr. Bernhard Schu
TITLE OF PERSON SIGNING Chief Executive Officer
ADDRESS OF PERSON SIGNING Kohlgasse 25, 89073 Ulm / GERMANY

Signature _____ Date 21/02/2001

Bioinfact GmbH
Loherstraße 18/1
89081 Ulm

SEQUENCE LISTING

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                165                 170                 175

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
            180                 185                 190

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
        195                 200                 205

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
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acggtgggag gtctatataa gcagagctgg tttagtgaac cgtcagatcc gctagcgcta 600

ccggtcgcca cc atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg 651
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val
1 5 10

ccc atc ctg gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc 699
Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser
15 20 25

gtg tcc ggc gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg 747
Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu
30 35 40 45

aag ttc atc tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc 795
Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu
50 55 60

gtg acc acc ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac 843
Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp
65 70 75

cac atg aag cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac 891
His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr
80 85 90

gtc cag gag cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc 939
Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr
95 100 105

cgc gcc gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag 987
Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu
110 115 120 125

ctg aag ggc atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag 1035
Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys
130 135 140

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ctg gag tac aac tac aac agc cac aac gtc tat atc atg gcc gac aag   1083
Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys
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cag aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag   1131
Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu
        160                 165                 170

gac ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc   1179
Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
    175                 180                 185

ggc gac ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag   1227
Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln
190                 195                 200                 205

tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg   1275
Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu
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ctg gag ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg   1323
Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu
            225                 230                 235

tac aag tcc gga ctc aga tcc atg tcc gag ccc aag gca att gat ccc   1371
Tyr Lys Ser Gly Leu Arg Ser Met Ser Glu Pro Lys Ala Ile Asp Pro
        240                 245                 250

aag ttg tcg acg acc gac agg gtg gtg aaa gct gtt cca ttt cct cca   1419
Lys Leu Ser Thr Thr Asp Arg Val Val Lys Ala Val Pro Phe Pro Pro
    255                 260                 265

agt cac cgg ctt aca gca aaa gaa gtg ttt gat aat gat gga aaa cct   1467
Ser His Arg Leu Thr Ala Lys Glu Val Phe Asp Asn Asp Gly Lys Pro
270                 275                 280                 285

cgt gtg gat atc tta aag gcg cat ctt atg aag gag gga agg ctg gaa   1515
Arg Val Asp Ile Leu Lys Ala His Leu Met Lys Glu Gly Arg Leu Glu
                290                 295                 300

gag agt gtt gca ttg aga ata ata aca gag ggt gca tca att ctt cga   1563
Glu Ser Val Ala Leu Arg Ile Ile Thr Glu Gly Ala Ser Ile Leu Arg
            305                 310                 315

cag gaa aaa aat ttg ctg gat att gat gcg cca gtc act gtt tgt ggg   1611
Gln Glu Lys Asn Leu Leu Asp Ile Asp Ala Pro Val Thr Val Cys Gly
        320                 325                 330

gac att cat gga caa ttc ttt gat ttg atg aag ctc ttt gaa gtc ggg   1659
Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly
    335                 340                 345

gga tct cct gcc aac act cgc tac ctc ttc tta ggg gac tat gtt gac   1707
Gly Ser Pro Ala Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp
350                 355                 360                 365

aga ggg tac ttc agt att gaa tgt gtg ctg tat ttg tgg gcc ttg aaa   1755
Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu Trp Ala Leu Lys
                370                 375                 380

att ctc tac ccc aaa aca ctg ttt tta ctt cgt gga aat cat gaa tgt   1803
Ile Leu Tyr Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys
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aga cat cta aca gag tat ttc aca ttt aaa caa gaa tgt aaa ata aag   1851
Arg His Leu Thr Glu Tyr Phe Thr Phe Lys Gln Glu Cys Lys Ile Lys
        400                 405                 410

tat tca gaa cga gta tat gat gcc tgt atg gat gcc ttt gac tgc ctt   1899
Tyr Ser Glu Arg Val Tyr Asp Ala Cys Met Asp Ala Phe Asp Cys Leu
    415                 420                 425

ccc ctg gct gcc ctg atg aac caa cag ttc ctg tgt gtg cat ggt ggt   1947
Pro Leu Ala Ala Leu Met Asn Gln Gln Phe Leu Cys Val His Gly Gly
430                 435                 440                 445

ttg tct cca gag att aac act tta gat gat atc aga aaa tta gac cga   1995
Leu Ser Pro Glu Ile Asn Thr Leu Asp Asp Ile Arg Lys Leu Asp Arg
                450                 455                 460

ttc aaa gaa cca cct gca tat gga cct atg tgt gat atc ctg tgg tca   2043
Phe Lys Glu Pro Pro Ala Tyr Gly Pro Met Cys Asp Ile Leu Trp Ser
            465                 470                 475

gac ccc ctg gaa gat ttt gga aat gag aag act cag gaa cat ttc act   2091
Asp Pro Leu Glu Asp Phe Gly Asn Glu Lys Thr Gln Glu His Phe Thr
        480                 485                 490

cac aac aca gtc agg ggg tgt tca tac ttc tac agt tac ccg gct gta   2139
His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala Val
    495                 500                 505

tgt gaa ttc tta cag cac aat aac ttg tta tct ata ctc cga gcc cac   2187
Cys Glu Phe Leu Gln His Asn Asn Leu Leu Ser Ile Leu Arg Ala His
510                 515                 520                 525

gaa gcc caa gat gca ggg tac cgc atg tac agg aaa agc caa aca aca   2235
Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Thr Thr
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ggc ttc cct tct cta att aca att ttt tca gca cca aat tac tta gat   2283
Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp
            545                 550                 555

gta tac aat aac aaa gct gca gta ttg aag tat gag aac aat gtt atg   2331
Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met
        560                 565                 570

aat atc agg caa ttc aac tgt tct cct cat cca tac tgg ctt cca aat   2379
Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn
    575                 580                 585

ttc atg gat gtt ttt act tgg tcc ctt cca ttt gtt ggg gaa aaa gtg   2427
Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val
590                 595                 600                 605

act gag atg ctg gta aat gtc ctc aac atc tgc tca gat gat gaa cta   2475
Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu Leu
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ggg tca gaa gaa gat gga ttt gat ggt gca aca gct gca gcc cgg aaa   2523
Gly Ser Glu Glu Asp Gly Phe Asp Gly Ala Thr Ala Ala Ala Arg Lys
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Glu Val Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val
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ttc tca gtg ctc aga gaa gag agt gag agt gtg ctg acg ctg aaa ggc   2619
Phe Ser Val Leu Arg Glu Glu Ser Glu Ser Val Leu Thr Leu Lys Gly
    655                 660                 665

ttg acc cca act ggc atg ctc ccc agc gga gta ctt tct gga ggg aag   2667
Leu Thr Pro Thr Gly Met Leu Pro Ser Gly Val Leu Ser Gly Gly Lys
670                 675                 680                 685

caa acc ctg caa agc gct act gtt gag gct att gag gct gat gaa gct   2715
Gln Thr Leu Gln Ser Ala Thr Val Glu Ala Ile Glu Ala Asp Glu Ala
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atc aaa gga ttt tca cca caa cat aag atc act agc ttc gag gaa gcc   2763
Ile Lys Gly Phe Ser Pro Gln His Lys Ile Thr Ser Phe Glu Glu Ala
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aag ggc tta gac cga att aat gag agg atg ccg cct cgc aga gat gcc   2811
Lys Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Arg Asp Ala
        720                 725                 730

atg ccc tct gac gcc aac ctt aac tcc atc aac aag gct ctc acc tca   2859
Met Pro Ser Asp Ala Asn Leu Asn Ser Ile Asn Lys Ala Leu Thr Ser
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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
         35                  40                  45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
     50                  55                  60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65                  70                  75                  80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
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Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
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Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
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Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
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Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
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Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
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Asp Ile Leu Lys Ala His Leu Met Lys Glu Gly Arg Leu Glu Glu Ser
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Val Ala Leu Arg Ile Ile Thr Glu Gly Ala Ser Ile Leu Arg Gln Glu
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Lys Asn Leu Leu Asp Ile Asp Ala Pro Val Thr Val Cys Gly Asp Ile
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His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser
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Pro Ala Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly
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Tyr Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg His
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165 170 175

Glu Arg Val Tyr Asp Ala Cys Met Asp Ala Phe Asp Cys Leu Pro Leu
180 185 190

Ala Ala Leu Met Asn Gln Gln Phe Leu Cys Val His Gly Gly Leu Ser
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Pro Glu Ile Asn Thr Leu Asp Asp Ile Arg Lys Leu Asp Arg Phe Lys
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Glu Pro Pro Ala Tyr Gly Pro Met Cys Asp Ile Leu Trp Ser Asp Pro
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245 250 255

Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala Val Cys Glu
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Phe Leu Gln His Asn Asn Leu Leu Ser Ile Leu Arg Ala His Glu Ala
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Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Thr Thr Gly Phe
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Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr
305 310 315 320

Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn Ile
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Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe Met
340 345 350

Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu
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Met Leu Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu Leu Gly Ser
370 375 380

Glu Glu Asp Gly Phe Asp Gly Ala Thr Ala Ala Ala Arg Lys Glu Val
385 390 395 400

Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser
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Val Leu Arg Glu Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr
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Pro Thr Gly Met Leu Pro Ser Gly Val Leu Ser Gly Gly Lys Gln Thr
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Leu Gln Ser Ala Thr Val Glu Ala Ile Glu Ala Asp Glu Ala Ile Lys
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Gly Phe Ser Pro Gln His Lys Ile Thr Ser Phe Glu Glu Ala Lys Gly
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Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu
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Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu
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Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp
65 70 75

cac atg aag cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac 891
His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr
80 85 90

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cgc gcc gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag 987
Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu
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130 135 140

ctg gag tac aac tac aac agc cac aac gtc tat atc atg gcc gac aag 1083

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Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys
            145                 150                 155

cag aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag   1131
Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu
        160                 165                 170

gac ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc   1179
Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
    175                 180                 185

ggc gac ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag   1227
Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln
190                 195                 200                 205

tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg   1275
Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu
                210                 215                 220

ctg gag ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg   1323
Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu
            225                 230                 235

tac aag tcc gga ctc aga tcc gcc gcc ccg gag ccg gcc cgg gct gca   1371
Tyr Lys Ser Gly Leu Arg Ser Ala Ala Pro Glu Pro Ala Arg Ala Ala
        240                 245                 250

ccg ccc cca ccc ccg ccc ccg ccg ccc cct ccc ggg gct gac cgc gtc   1419
Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Gly Ala Asp Arg Val
    255                 260                 265

gtc aaa gct gtc cct ttc ccc cca aca cat cgc ttg aca tct gaa gaa   1467
Val Lys Ala Val Pro Phe Pro Pro Thr His Arg Leu Thr Ser Glu Glu
270                 275                 280                 285

gta ttt gat ttg gat ggg ata ccc agg gtt gat gtt ctg aag aac cac   1515
Val Phe Asp Leu Asp Gly Ile Pro Arg Val Asp Val Leu Lys Asn His
                290                 295                 300

ttg gtg aaa gaa ggt cga gta gat gaa gaa att gcg ctt aga att atc   1563
Leu Val Lys Glu Gly Arg Val Asp Glu Glu Ile Ala Leu Arg Ile Ile
            305                 310                 315

aat gag ggt gct gcc atc ctt cgg aga gag aaa acc atg ata gaa gta   1611
Asn Glu Gly Ala Ala Ile Leu Arg Arg Glu Lys Thr Met Ile Glu Val
        320                 325                 330

gaa gct cca atc aca gtg tgt ggt gac atc cat ggc caa ttt ttt gat   1659
Glu Ala Pro Ile Thr Val Cys Gly Asp Ile His Gly Gln Phe Phe Asp
    335                 340                 345

ctg atg aaa ctt ttt gaa gta gga gga tca cct gct aat aca cga tac   1707
Leu Met Lys Leu Phe Glu Val Gly Gly Ser Pro Ala Asn Thr Arg Tyr
350                 355                 360                 365

ctt ttt ctt ggc gat tat gtg gac aga ggt tat ttt agt ata gag tgt   1755
Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly Tyr Phe Ser Ile Glu Cys
                370                 375                 380

gtc tta tat tta tgg gtt ctg aag att cta tac cca agc aca tta ttt   1803
Val Leu Tyr Leu Trp Val Leu Lys Ile Leu Tyr Pro Ser Thr Leu Phe
            385                 390                 395
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ctt ctg aga ggc aac cat gaa tgc aga cac ctt act gaa tat ttt acc   1851
Leu Leu Arg Gly Asn His Glu Cys Arg His Leu Thr Glu Tyr Phe Thr
        400                 405                 410

ttt aag cag gaa tgt aaa att aag tat tcg gaa aga gtc tat gaa gct   1899
Phe Lys Gln Glu Cys Lys Ile Lys Tyr Ser Glu Arg Val Tyr Glu Ala
    415                 420                 425

tgt atg gaa gct ttt gat agt ttg cct ctt gct gca ctt tta aac caa   1947
Cys Met Glu Ala Phe Asp Ser Leu Pro Leu Ala Ala Leu Leu Asn Gln
430                 435                 440                 445

cag ttt ctt tgt gtt cat ggt gga ctt tca cca gaa ata cac aca ctg   1995
Gln Phe Leu Cys Val His Gly Gly Leu Ser Pro Glu Ile His Thr Leu
                450                 455                 460

gat gat att agg aga tta gat aga ttc aaa gag cca cct gca ttt gga   2043
Asp Asp Ile Arg Arg Leu Asp Arg Phe Lys Glu Pro Pro Ala Phe Gly
            465                 470                 475

cca atg tgt gac ttg tta tgg tcc gat cct tct gaa gat ttt gga aat   2091
Pro Met Cys Asp Leu Leu Trp Ser Asp Pro Ser Glu Asp Phe Gly Asn
        480                 485                 490

gaa aaa tca cag gaa cat ttt agt cac aat aca gtt cga gga tgt tct   2139
Glu Lys Ser Gln Glu His Phe Ser His Asn Thr Val Arg Gly Cys Ser
    495                 500                 505

tat ttt tat aac tat cca gca gtg tgt gaa ttt ttg caa aac aat aat   2187
Tyr Phe Tyr Asn Tyr Pro Ala Val Cys Glu Phe Leu Gln Asn Asn Asn
510                 515                 520                 525

ttg tta tcg att att aga gct cat gaa gct caa gat gca ggc tat aga   2235
Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg
                530                 535                 540

atg tac aga aaa agt caa act aca ggg ttc cct tca tta ata aca att   2283
Met Tyr Arg Lys Ser Gln Thr Thr Gly Phe Pro Ser Leu Ile Thr Ile
            545                 550                 555

ttt tcg gca cct aat tac tta gat gtc tac aat aat aaa gct gct gta   2331
Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr Asn Asn Lys Ala Ala Val
        560                 565                 570

tta aag tat gaa aat aat gtg atg aat att cga cag ttt aac tgt tct   2379
Leu Lys Tyr Glu Asn Asn Val Met Asn Ile Arg Gln Phe Asn Cys Ser
    575                 580                 585

cca cat cct tac tgg ttg cct aat ttt atg gat gtc ttc acg tgg tct   2427
Pro His Pro Tyr Trp Leu Pro Asn Phe Met Asp Val Phe Thr Trp Ser
590                 595                 600                 605

tta ccg ttt gtt gga gaa aaa gtg aca gaa atg ttg gta aat gtt ctg   2475
Leu Pro Phe Val Gly Glu Lys Val Thr Glu Met Leu Val Asn Val Leu
                610                 615                 620

agt att tgc tct gat gat gaa cta atg act gaa ggt gaa gac cag ttt   2523
Ser Ile Cys Ser Asp Asp Glu Leu Met Thr Glu Gly Glu Asp Gln Phe
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gat ggt tca gct gca gcc cgg aaa gaa atc ata aga aac aaa att cga   2571
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Asp Gly Ser Ala Ala Ala Arg Lys Glu Ile Ile Arg Asn Lys Ile Arg
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gca att ggc aag atg gca aga gtc ttc tct gtt ctc agg gag gag agt   2619
Ala Ile Gly Lys Met Ala Arg Val Phe Ser Val Leu Arg Glu Glu Ser
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gaa agt gtg ctg aca ctc aag ggc ctg act ccc aca ggg atg ttg cct   2667
Glu Ser Val Leu Thr Leu Lys Gly Leu Thr Pro Thr Gly Met Leu Pro
670                 675                 680                 685

agt gga gtg tta gct gga gga cgg cag acc ctg caa agt gcc aca gtt   2715
Ser Gly Val Leu Ala Gly Gly Arg Gln Thr Leu Gln Ser Ala Thr Val
                690                 695                 700

gag gct att gag gct gaa aaa gca ata cga gga ttc tct cca cca cat   2763
Glu Ala Ile Glu Ala Glu Lys Ala Ile Arg Gly Phe Ser Pro Pro His
            705                 710                 715

aga atc tgc agt ttt gaa gag gca aag ggt ttg gat agg atc aat gag   2811
Arg Ile Cys Ser Phe Glu Glu Ala Lys Gly Leu Asp Arg Ile Asn Glu
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aga atg cca cct cgg aaa gat gct gta cag caa gat ggt ttc aat tct   2859
Arg Met Pro Pro Arg Lys Asp Ala Val Gln Gln Asp Gly Phe Asn Ser
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ctg aac acc gca cat gcc act gag aac cac ggg acg ggc aac cat act   2907
Leu Asn Thr Ala His Ala Thr Glu Asn His Gly Thr Gly Asn His Thr
750                 755                 760                 765

gcc cag tga ttaactaggg taccccggga tccaccggat ctagataact           2956
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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
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Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
     50                  55                  60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
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Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
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Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
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Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
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Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
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Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
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Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
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Asp Leu Asp Gly Ile Pro Arg Val Asp Val Leu Lys Asn His Leu Val
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Lys Glu Gly Arg Val Asp Glu Glu Ile Ala Leu Arg Ile Ile Asn Glu
65 70 75 80

Gly Ala Ala Ile Leu Arg Arg Glu Lys Thr Met Ile Glu Val Glu Ala
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Pro Ile Thr Val Cys Gly Asp Ile His Gly Gln Phe Phe Asp Leu Met
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Lys Leu Phe Glu Val Gly Gly Ser Pro Ala Asn Thr Arg Tyr Leu Phe
115 120 125

Leu Gly Asp Tyr Val Asp Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu
130 135 140

Tyr Leu Trp Val Leu Lys Ile Leu Tyr Pro Ser Thr Leu Phe Leu Leu
145 150 155 160

Arg Gly Asn His Glu Cys Arg His Leu Thr Glu Tyr Phe Thr Phe Lys
165 170 175

Gln Glu Cys Lys Ile Lys Tyr Ser Glu Arg Val Tyr Glu Ala Cys Met
180 185 190

Glu Ala Phe Asp Ser Leu Pro Leu Ala Ala Leu Leu Asn Gln Gln Phe
195 200 205

Leu Cys Val His Gly Gly Leu Ser Pro Glu Ile His Thr Leu Asp Asp
210 215 220

Ile Arg Arg Leu Asp Arg Phe Lys Glu Pro Pro Ala Phe Gly Pro Met

225 230 235 240

Cys Asp Leu Leu Trp Ser Asp Pro Ser Glu Asp Phe Gly Asn Glu Lys
245 250 255

Ser Gln Glu His Phe Ser His Asn Thr Val Arg Gly Cys Ser Tyr Phe
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Tyr Asn Tyr Pro Ala Val Cys Glu Phe Leu Gln Asn Asn Asn Leu Leu
275 280 285

Ser Ile Ile Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr
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Arg Lys Ser Gln Thr Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser
305 310 315 320

Ala Pro Asn Tyr Leu Asp Val Tyr Asn Asn Lys Ala Ala Val Leu Lys
325 330 335

Tyr Glu Asn Asn Val Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His
340 345 350

Pro Tyr Trp Leu Pro Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro
355 360 365

Phe Val Gly Glu Lys Val Thr Glu Met Leu Val Asn Val Leu Ser Ile
370 375 380

Cys Ser Asp Asp Glu Leu Met Thr Glu Gly Glu Asp Gln Phe Asp Gly
385 390 395 400

Ser Ala Ala Ala Arg Lys Glu Ile Ile Arg Asn Lys Ile Arg Ala Ile
405 410 415

Gly Lys Met Ala Arg Val Phe Ser Val Leu Arg Glu Glu Ser Glu Ser
420 425 430

Val Leu Thr Leu Lys Gly Leu Thr Pro Thr Gly Met Leu Pro Ser Gly
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Val Leu Ala Gly Gly Arg Gln Thr Leu Gln Ser Ala Thr Val Glu Ala
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Ile Glu Ala Glu Lys Ala Ile Arg Gly Phe Ser Pro Pro His Arg Ile
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Cys Ser Phe Glu Glu Ala Lys Gly Leu Asp Arg Ile Asn Glu Arg Met
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Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu
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Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu
50 55 60

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Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp
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80 85 90

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cgc gcc gag gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag 987
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110                 115                 120                 125

ctg aag ggc atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag   1035
Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys
                130                 135                 140

ctg gag tac aac tac aac agc cac aac gtc tat atc atg gcc gac aag   1083
Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys
            145                 150                 155

cag aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag   1131
Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu
        160                 165                 170

gac ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc   1179
Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
    175                 180                 185

ggc gac ggc ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag   1227
Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln
190                 195                 200                 205

tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg   1275
Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu
                210                 215                 220

ctg gag ttc gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg   1323
Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu
            225                 230                 235

tac aag tcc gga ctc aga tcc aga tcc atg tcc ggg agg cgc ttc cac   1371
Tyr Lys Ser Gly Leu Arg Ser Arg Ser Met Ser Gly Arg Arg Phe His
        240                 245                 250

ctc tcc acc acc gac cgc gtc atc aaa gct gtc ccc ttt cct cca acc   1419
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    255                 260                 265

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Gln Arg Leu Thr Phe Lys Glu Val Phe Glu Asn Gly Lys Pro Lys Val
270                 275                 280                 285

gat gtt tta aaa aac cat ttg gta aag gaa gga cga ctg gaa gag gaa   1515
Asp Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Leu Glu Glu Glu
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gta gcc tta aag ata atc aat gat ggg gct gcc atc ctg agg caa gag   1563
Val Ala Leu Lys Ile Ile Asn Asp Gly Ala Ala Ile Leu Arg Gln Glu
            305                 310                 315

aag act atg ata gaa gta gat gct cca atc aca gta tgt ggt gat att   1611
Lys Thr Met Ile Glu Val Asp Ala Pro Ile Thr Val Cys Gly Asp Ile
        320                 325                 330

cat gga caa ttc ttt gac cta atg aag tta ttt gaa gtt gga gga tca   1659
His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser
    335                 340                 345

cct agt aac aca cgc tac ctc ttt ctg ggt gac tat gtg gac aga ggc   1707
Pro Ser Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly
350                 355                 360                 365
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                370                 375                 380

cat ccc aaa aca ttg ttt ctg ctt cgg gga aat cat gaa tgc agg cat   1803
His Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg His
            385                 390                 395

ctt aca gac tat ttc acc ttc aaa cag gaa tgt cga atc aaa tat tcg   1851
Leu Thr Asp Tyr Phe Thr Phe Lys Gln Glu Cys Arg Ile Lys Tyr Ser
        400                 405                 410

gaa cag gtg tat gat gcc tgt atg gag aca ttt gac tgt ctt cct ctt   1899
Glu Gln Val Tyr Asp Ala Cys Met Glu Thr Phe Asp Cys Leu Pro Leu
    415                 420                 425

gct gcc ctc tta aac cag cag ttt ctc tgt gta cat gga gga atg tca   1947
Ala Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly Met Ser
430                 435                 440                 445

cct gaa att act tct tta gat gac att agg aaa tta gac agg ttt acg   1995
Pro Glu Ile Thr Ser Leu Asp Asp Ile Arg Lys Leu Asp Arg Phe Thr
                450                 455                 460

gaa cct ccc gcc ttt gga cct gtg tgt gac ctg ctt tgg tct gat ccc   2043
Glu Pro Pro Ala Phe Gly Pro Val Cys Asp Leu Leu Trp Ser Asp Pro
            465                 470                 475

tca gag gat tat ggc aat gag aag acc ttg gag cac tat acc cac aac   2091
Ser Glu Asp Tyr Gly Asn Glu Lys Thr Leu Glu His Tyr Thr His Asn
        480                 485                 490

act gtc cga ggg tgc tct tat ttc tac agt tac cct gca gtt tgt gaa   2139
Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala Val Cys Glu
    495                 500                 505

ttt ttg cag aac aat aat tta cta tca att atc aga gcc cat gaa gcc   2187
Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His Glu Ala
510                 515                 520                 525

caa gat gct ggg tat cga atg tac agg aag agc caa gcc aca ggc ttt   2235
Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Ala Thr Gly Phe
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cca tca ctt att aca att ttc tct gcc ccc aat tac cta gat gtc tat   2283
Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr
            545                 550                 555

aac aat aaa gct gct gtg ttg aaa tat gaa aac aat gtc atg aat atc   2331
Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn Ile
        560                 565                 570

agg cag ttt aac tgt tct cca cac ccc tac tgg ctt cca aac ttt atg   2379
Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe Met
    575                 580                 585

gat gtt ttc aca tgg tct ttg cct ttt gtt ggg gaa aaa gtc aca gag   2427
Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu
590                 595                 600                 605

atg ctg gta aat gtg ctc aac ata tgc tct gat gac gaa ctg att tct   2475
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gat gat gaa gca gaa gga agc act aca gtt cgt aag gag atc atc agg   2523
Asp Asp Glu Ala Glu Gly Ser Thr Thr Val Arg Lys Glu Ile Ile Arg
            625                 630                 635

aat aag atc aga gcc att ggg aag atg gca cgg gtc ttt tca att ctt   2571
Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser Ile Leu
        640                 645                 650

cgg caa gaa agt gag agt gtg ctg act ctc aag ggc ctg act ccc aca   2619
Arg Gln Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr Pro Thr
    655                 660                 665

ggc aca ctc cct ctg ggc gtc ctc tca gga ggc aag cag act atc gag   2667
Gly Thr Leu Pro Leu Gly Val Leu Ser Gly Gly Lys Gln Thr Ile Glu
670                 675                 680                 685

aca gcc atc aga ggg ttc tcg ctt cag cac aag atc cgg agt ttt gaa   2715
Thr Ala Ile Arg Gly Phe Ser Leu Gln His Lys Ile Arg Ser Phe Glu
                690                 695                 700

gaa gcg cga ggt ctg gac cga att aat gag cga atg cca ccc cga aag   2763
Glu Ala Arg Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys
            705                 710                 715

gat agc ata tac cct ggt ggg cca atg aaa tct gta acc tca gca cac   2811
Asp Ser Ile Tyr Pro Gly Gly Pro Met Lys Ser Val Thr Ser Ala His
        720                 725                 730

tca cat gct gcg cac agg agc gac caa ggg aag aaa gcc cat tca       2856
Ser His Ala Ala His Arg Ser Asp Gln Gly Lys Lys Ala His Ser
    735                 740                 745

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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
         35                  40                  45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
     50                  55                  60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65                  70                  75                  80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                 85                  90                  95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
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180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
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35 40 45

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50 55 60

Leu Lys Ile Ile Asn Asp Gly Ala Ala Ile Leu Arg Gln Glu Lys Thr
65 70 75 80

Met Ile Glu Val Asp Ala Pro Ile Thr Val Cys Gly Asp Ile His Gly
85 90 95

Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser Pro Ser
100 105 110

Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly Tyr Phe
115 120 125

Ser Ile Glu Cys Val Leu Tyr Leu Trp Ser Leu Lys Ile Asn His Pro
130 135 140

Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg His Leu Thr
145 150 155 160

Asp Tyr Phe Thr Phe Lys Gln Glu Cys Arg Ile Lys Tyr Ser Glu Gln
165 170 175

Val Tyr Asp Ala Cys Met Glu Thr Phe Asp Cys Leu Pro Leu Ala Ala
180 185 190

Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly Met Ser Pro Glu
195 200 205

Ile Thr Ser Leu Asp Asp Ile Arg Lys Leu Asp Arg Phe Thr Glu Pro
210 215 220

Pro Ala Phe Gly Pro Val Cys Asp Leu Leu Trp Ser Asp Pro Ser Glu
225 230 235 240

Asp Tyr Gly Asn Glu Lys Thr Leu Glu His Tyr Thr His Asn Thr Val
245 250 255

Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala Val Cys Glu Phe Leu
260 265 270

Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp
275 280 285

Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Ala Thr Gly Phe Pro Ser
290 295 300

Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr Asn Asn
305 310 315 320

Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn Ile Arg Gln
325 330 335

Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe Met Asp Val
340 345 350

Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu Met Leu
355 360 365

Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu Leu Ile Ser Asp Asp
370 375 380

Glu Ala Glu Gly Ser Thr Thr Val Arg Lys Glu Ile Ile Arg Asn Lys
385 390 395 400

Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser Ile Leu Arg Gln
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Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr Pro Thr Gly Thr
420 425 430

Leu Pro Leu Gly Val Leu Ser Gly Gly Lys Gln Thr Ile Glu Thr Ala
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Ile Arg Gly Phe Ser Leu Gln His Lys Ile Arg Ser Phe Glu Glu Ala
450 455 460

Arg Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ser
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Ala Thr Lys Ala Val Cys Val Leu
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aag ggc gac ggc cca gtg cag ggc atc atc aat ttc gag cag aag gaa 222
Lys Gly Asp Gly Pro Val Gln Gly Ile Ile Asn Phe Glu Gln Lys Glu
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Ser Asn Gly Pro Val Lys Val Trp Gly Ser Ile Lys Gly Leu Thr Glu
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Gly Leu His Gly Phe His Val His Glu Phe Gly Asp Asn Thr Ala Gly
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tgt acc agt gca ggt cct cac ttt aat cct cta tcc aga aaa cac ggt 366
Cys Thr Ser Ala Gly Pro His Phe Asn Pro Leu Ser Arg Lys His Gly
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ggg cca aag gat gaa gag agg cat gtt gga gac ttg ggc aat gtg act 414
Gly Pro Lys Asp Glu Glu Arg His Val Gly Asp Leu Gly Asn Val Thr
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gct gac aaa gat ggt gtg gcc gat gtg tct att gaa gat tct gtg atc 462
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Asn Pro Leu Ser Arg Lys His Gly Gly Pro Lys Asp Glu Glu Arg His
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Val Gly Asp Leu Gly Asn Val Thr Ala Asp Lys Asp Gly Val Ala Asp
                 85                  90                  95

Val Ser Ile Glu Asp Ser Val Ile Ser Leu Ser Gly Asp His Cys Ile
            100                 105                 110

Ile Gly Arg Thr Leu Val Val His Glu Lys Ala Asp Asp Leu Gly Lys
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Ala Cys Gly Val Ile Gly Ile Ala Gln
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gcg acg aag gcc gtg tgc gtg ctg aag ggc gac ggc cca gtg cag ggc 165
Ala Thr Lys Ala Val Cys Val Leu Lys Gly Asp Gly Pro Val Gln Gly
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atc atc aat ttc gag cag aag gaa agt aat gga cca gtg aag gtg tgg 213
Ile Ile Asn Phe Glu Gln Lys Glu Ser Asn Gly Pro Val Lys Val Trp
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gga agc att aaa gga ctg act gaa ggc ctg cat gga ttc cat gtt cat   261
Gly Ser Ile Lys Gly Leu Thr Glu Gly Leu His Gly Phe His Val His
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gag ttt gga gat aat aca gca ggc tgt acc agt gca ggt cct cac ttt   309
Glu Phe Gly Asp Asn Thr Ala Gly Cys Thr Ser Ala Gly Pro His Phe
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aat cct cta tcc aga aaa cac ggt ggg cca aag gat gaa gag agg cat   357
Asn Pro Leu Ser Arg Lys His Gly Gly Pro Lys Asp Glu Glu Arg His
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gtt gga gac ttg ggc aat gtg act gct gac aaa gat ggt gtg gcc gat   405
Val Gly Asp Leu Gly Asn Val Thr Ala Asp Lys Asp Gly Val Ala Asp
             85                  90                  95

gtg tct att gaa gat tct gtg atc tca ctc tca gga gac cat tgc atc   453
Val Ser Ile Glu Asp Ser Val Ile Ser Leu Ser Gly Asp His Cys Ile
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att ggc cgc aca ctg gtg gtc cat gaa aaa gca gat gac ttg ggc aaa   501
Ile Gly Arg Thr Leu Val Val His Glu Lys Ala Asp Asp Leu Gly Lys
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ggt gga aat gaa gaa agt aca aag aca gga aac gct gga agt cgt ttg   549
Gly Gly Asn Glu Glu Ser Thr Lys Thr Gly Asn Ala Gly Ser Arg Leu
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gct tgt ggt gta att ggg atc gcc caa agatctcatc accatcacca         596
Ala Cys Gly Val Ile Gly Ile Ala Gln
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Trp Gly Ser Ile Lys Gly Leu Thr Glu Gly Leu His Gly Phe His Val
         35                  40                  45

His Glu Phe Gly Asp Asn Thr Ala Gly Cys Thr Ser Ala Gly Pro His
     50                  55                  60

Phe Asn Pro Leu Ser Arg Lys His Gly Gly Pro Lys Asp Glu Glu Arg
 65                  70                  75                  80

His Val Gly Asp Leu Gly Asn Val Thr Ala Asp Lys Asp Gly Val Ala
                 85                  90                  95

Asp Val Ser Ile Glu Asp Ser Val Ile Ser Leu Ser Gly Asp His Cys
            100                 105                 110

Ile Ile Gly Arg Thr Leu Val Val His Glu Lys Ala Asp Asp Leu Gly
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Leu Ala Cys Gly Val Ile Gly Ile Ala Gln
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Met
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aga gga tcg cat cac cat cac cat cac gga tcc atg tcc gag ccc aag 165
Arg Gly Ser His His His His His His Gly Ser Met Ser Glu Pro Lys
5 10 15

gca att gat ccc aag ttg tcg acg acc gac agg gtg gtg aaa gct gtt 213
Ala Ile Asp Pro Lys Leu Ser Thr Thr Asp Arg Val Val Lys Ala Val
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cca ttt cct cca agt cac cgg ctt aca gca aaa gaa gtg ttt gat aat 261
Pro Phe Pro Pro Ser His Arg Leu Thr Ala Lys Glu Val Phe Asp Asn
35 40 45

gat gga aaa cct cgt gtg gat atc tta aag gcg cat ctt atg aag gag 309
Asp Gly Lys Pro Arg Val Asp Ile Leu Lys Ala His Leu Met Lys Glu
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gga agg ctg gaa gag agt gtt gca ttg aga ata ata aca gag ggt gca 357
Gly Arg Leu Glu Glu Ser Val Ala Leu Arg Ile Ile Thr Glu Gly Ala
70 75 80

tca att ctt cga cag gaa aaa aat ttg ctg gat att gat gcg cca gtc 405
Ser Ile Leu Arg Gln Glu Lys Asn Leu Leu Asp Ile Asp Ala Pro Val
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Thr Val Cys Gly Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu
100 105 110

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Phe Glu Val Gly Gly Ser Pro Ala Asn Thr Arg Tyr Leu Phe Leu Gly
115 120 125

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gac tat gtt gac aga ggg tac ttc agt att gaa tgt gtg ctg tat ttg   549
Asp Tyr Val Asp Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu
130                 135                 140                 145

tgg gcc ttg aaa att ctc tac ccc aaa aca ctg ttt tta ctt cgt gga   597
Trp Ala Leu Lys Ile Leu Tyr Pro Lys Thr Leu Phe Leu Leu Arg Gly
                150                 155                 160

aat cat gaa tgt aga cat cta aca gag tat ttc aca ttt aaa caa gaa   645
Asn His Glu Cys Arg His Leu Thr Glu Tyr Phe Thr Phe Lys Gln Glu
            165                 170                 175

tgt aaa ata aag tat tca gaa cga gta tat gat gcc tgt atg gat gcc   693
Cys Lys Ile Lys Tyr Ser Glu Arg Val Tyr Asp Ala Cys Met Asp Ala
        180                 185                 190

ttt gac tgc ctt ccc ctg gct gcc ctg atg aac caa cag ttc ctg tgt   741
Phe Asp Cys Leu Pro Leu Ala Ala Leu Met Asn Gln Gln Phe Leu Cys
    195                 200                 205

gtg cat ggt ggt ttg tct cca gag att aac act tta gat gat atc aga   789
Val His Gly Gly Leu Ser Pro Glu Ile Asn Thr Leu Asp Asp Ile Arg
210                 215                 220                 225

aaa tta gac cga ttc aaa gaa cca cct gca tat gga cct atg tgt gat   837
Lys Leu Asp Arg Phe Lys Glu Pro Pro Ala Tyr Gly Pro Met Cys Asp
                230                 235                 240

atc ctg tgg tca gac ccc ctg gaa gat ttt gga aat gag aag act cag   885
Ile Leu Trp Ser Asp Pro Leu Glu Asp Phe Gly Asn Glu Lys Thr Gln
            245                 250                 255

gaa cat ttc act cac aac aca gtc agg ggg tgt tca tac ttc tac agt   933
Glu His Phe Thr His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser
        260                 265                 270

tac ccg gct gta tgt gaa ttc tta cag cac aat aac ttg tta tct ata   981
Tyr Pro Ala Val Cys Glu Phe Leu Gln His Asn Asn Leu Leu Ser Ile
    275                 280                 285

ctc cga gcc cac gaa gcc caa gat gca ggg tac cgc atg tac agg aaa  1029
Leu Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys
290                 295                 300                 305

agc caa aca aca ggc ttc cct tct cta att aca att ttt tca gca cca  1077
Ser Gln Thr Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro
                310                 315                 320

aat tac tta gat gta tac aat aac aaa gct gca gta ttg aag tat gag  1125
Asn Tyr Leu Asp Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu
            325                 330                 335

aac aat gtt atg aat atc agg caa ttc aac tgt tct cct cat cca tac  1173
Asn Asn Val Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr
        340                 345                 350

tgg ctt cca aat ttc atg gat gtt ttt act tgg tcc ctt cca ttt gtt  1221
Trp Leu Pro Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val
    355                 360                 365

ggg gaa aaa gtg act gag atg ctg gta aat gtc ctc aac atc tgc tca  1269
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Gly Glu Lys Val Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser
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Asp Asp Glu Leu Gly Ser Glu Glu Asp Gly Phe Asp Gly Ala Thr Ala
                390                 395                 400

gca gcc cgg aaa gag gtg ata agg aac aag atc cga gca ata ggc aaa   1365
Ala Ala Arg Lys Glu Val Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys
            405                 410                 415

atg gcc aga gtg ttc tca gtg ctc aga gaa gag agt gag agt gtg ctg   1413
Met Ala Arg Val Phe Ser Val Leu Arg Glu Glu Ser Glu Ser Val Leu
        420                 425                 430

acg ctg aaa ggc ttg acc cca act ggc atg ctc ccc agc gga gta ctt   1461
Thr Leu Lys Gly Leu Thr Pro Thr Gly Met Leu Pro Ser Gly Val Leu
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tct gga ggg aag caa acc ctg caa agc gct act gtt gag gct att gag   1509
Ser Gly Gly Lys Gln Thr Leu Gln Ser Ala Thr Val Glu Ala Ile Glu
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gct gat gaa gct atc aaa gga ttt tca cca caa cat aag atc act agc   1557
Ala Asp Glu Ala Ile Lys Gly Phe Ser Pro Gln His Lys Ile Thr Ser
                470                 475                 480

ttc gag gaa gcc aag ggc tta gac cga att aat gag agg atg ccg cct   1605
Phe Glu Glu Ala Lys Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro
            485                 490                 495

cgc aga gat gcc atg ccc tct gac gcc aac ctt aac tcc atc aac aag   1653
Arg Arg Asp Ala Met Pro Ser Asp Ala Asn Leu Asn Ser Ile Asn Lys
        500                 505                 510

gct ctc acc tca gag act aac ggc acg gac agc aat ggc agt aat agc   1701
Ala Leu Thr Ser Glu Thr Asn Gly Thr Asp Ser Asn Gly Ser Asn Ser
    515                 520                 525

agc aat att cag tga ttaactaggg taccccgggg taccaaagag gagaaattaa   1756
Ser Asn Ile Gln
530

ct atg gga aat gag gca agt tat cct ttg gaa atg tgc tca cac ttt     1803
   Met Gly Asn Glu Ala Ser Tyr Pro Leu Glu Met Cys Ser His Phe
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gat gca gat gaa att aaa agg cta gga aag aga ttt aag aag ctc gat   1851
Asp Ala Asp Glu Ile Lys Arg Leu Gly Lys Arg Phe Lys Lys Leu Asp
550                 555                 560                 565

ttg gac aat tct ggt tct ttg agt gtg gaa gag ttc atg tct cta cct   1899
Leu Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Leu Pro
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gag tta caa cag aat ccc tta gta cag cga gta ata gat ata ttc gac   1947
Glu Leu Gln Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp
            585                 590                 595

aca gat ggg aat gga gaa gta gac ttt aaa gag ttc att gag gga gtc   1995
Thr Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val
        600                 605                 610
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tct cag ttc agt gtc aaa gga gat aag gaa cag aag ttg agg ttt gct   2043
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ttc cgt atc tat gac atg gat aaa gac ggc tat att tcc aat ggg gaa   2091
Phe Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu
630                 635                 640                 645

ctc ttc cag gtg cta aag atg atg gtg ggg aac aat ctg aaa gat aca   2139
Leu Phe Gln Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr
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cag tta cag caa att gta gac aaa acc ata ata aat gca gat aag gat   2187
Gln Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp
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ggg gat gga aga ata tcc ttt gaa gaa ttc tgt gct gtt gta ggc ggc   2235
Gly Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly
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cta gat atc cac aaa aag atg gtg gta gat gtg tga ttaattagaa        2281
Leu Asp Ile His Lys Lys Met Val Val Asp Val
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Lys	Ala	Ile	Asp	Pro	Lys	Leu	Ser	Thr	Thr	Asp	Arg	Val	Val	Lys	Ala
			20					25					30		
Val	Pro	Phe	Pro	Pro	Ser	His	Arg	Leu	Thr	Ala	Lys	Glu	Val	Phe	Asp
		35					40					45			
Asn	Asp	Gly	Lys	Pro	Arg	Val	Asp	Ile	Leu	Lys	Ala	His	Leu	Met	Lys
	50					55					60				
Glu	Gly	Arg	Leu	Glu	Glu	Ser	Val	Ala	Leu	Arg	Ile	Ile	Thr	Glu	Gly
65					70					75					80
Ala	Ser	Ile	Leu	Arg	Gln	Glu	Lys	Asn	Leu	Leu	Asp	Ile	Asp	Ala	Pro
				85					90					95	
Val	Thr	Val	Cys	Gly	Asp	Ile	His	Gly	Gln	Phe	Phe	Asp	Leu	Met	Lys
			100					105					110		
Leu	Phe	Glu	Val	Gly	Gly	Ser	Pro	Ala	Asn	Thr	Arg	Tyr	Leu	Phe	Leu
		115					120					125			
Gly	Asp	Tyr	Val	Asp	Arg	Gly	Tyr	Phe	Ser	Ile	Glu	Cys	Val	Leu	Tyr
	130					135					140				
Leu	Trp	Ala	Leu	Lys	Ile	Leu	Tyr	Pro	Lys	Thr	Leu	Phe	Leu	Leu	Arg
145					150					155					160
Gly	Asn	His	Glu	Cys	Arg	His	Leu	Thr	Glu	Tyr	Phe	Thr	Phe	Lys	Gln
				165					170					175	
Glu	Cys	Lys	Ile	Lys	Tyr	Ser	Glu	Arg	Val	Tyr	Asp	Ala	Cys	Met	Asp
			180					185					190		
Ala	Phe	Asp	Cys	Leu	Pro	Leu	Ala	Ala	Leu	Met	Asn	Gln	Gln	Phe	Leu
		195					200					205			
Cys	Val	His	Gly	Gly	Leu	Ser	Pro	Glu	Ile	Asn	Thr	Leu	Asp	Asp	Ile
	210					215					220				
Arg	Lys	Leu	Asp	Arg	Phe	Lys	Glu	Pro	Pro	Ala	Tyr	Gly	Pro	Met	Cys
225					230					235					240

Asp Ile Leu Trp Ser Asp Pro Leu Glu Asp Phe Gly Asn Glu Lys Thr
245 250 255

Gln Glu His Phe Thr His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr
260 265 270

Ser Tyr Pro Ala Val Cys Glu Phe Leu Gln His Asn Asn Leu Leu Ser
275 280 285

Ile Leu Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg
290 295 300

Lys Ser Gln Thr Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala
305 310 315 320

Pro Asn Tyr Leu Asp Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr
325 330 335

Glu Asn Asn Val Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro
340 345 350

Tyr Trp Leu Pro Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe
355 360 365

Val Gly Glu Lys Val Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys
370 375 380

Ser Asp Asp Glu Leu Gly Ser Glu Glu Asp Gly Phe Asp Gly Ala Thr
385 390 395 400

Ala Ala Ala Arg Lys Glu Val Ile Arg Asn Lys Ile Arg Ala Ile Gly
405 410 415

Lys Met Ala Arg Val Phe Ser Val Leu Arg Glu Glu Ser Glu Ser Val
420 425 430

Leu Thr Leu Lys Gly Leu Thr Pro Thr Gly Met Leu Pro Ser Gly Val
435 440 445

Leu Ser Gly Gly Lys Gln Thr Leu Gln Ser Ala Thr Val Glu Ala Ile
450 455 460

Glu Ala Asp Glu Ala Ile Lys Gly Phe Ser Pro Gln His Lys Ile Thr
465 470 475 480

Ser Phe Glu Glu Ala Lys Gly Leu Asp Arg Ile Asn Glu Arg Met Pro
485 490 495

Pro Arg Arg Asp Ala Met Pro Ser Asp Ala Asn Leu Asn Ser Ile Asn
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Lys Ala Leu Thr Ser Glu Thr Asn Gly Thr Asp Ser Asn Gly Ser Asn
515 520 525

Ser Ser Asn Ile Gln
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<213> Homo sapiens

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  1               5                  10                  15

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             20                  25                  30

Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Leu Pro Glu
         35                  40                  45

Leu Gln Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
     50                  55                  60

Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val Ser
 65                  70                  75                  80

Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
                 85                  90                  95

Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
            100                 105                 110

Phe Gln Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
        115                 120                 125

Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Gly
    130                 135                 140

Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly Leu
145                 150                 155                 160

Asp Ile His Lys Lys Met Val Val Asp Val
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                                                                  Met
                                                                   1

aga gga tcg cat cac cat cac cat cac gga tcc atg tcc gag ccc aag   165
Arg Gly Ser His His His His His His Gly Ser Met Ser Glu Pro Lys
               5                  10                  15

gca att gat ccc aag ttg tcg acg acc gac agg gtg gtg aaa gct gtt   213
Ala Ile Asp Pro Lys Leu Ser Thr Thr Asp Arg Val Val Lys Ala Val
          20                  25                  30

cca ttt cct cca agt cac cgg ctt aca gca aaa gaa gtg ttt gat aat   261
Pro Phe Pro Pro Ser His Arg Leu Thr Ala Lys Glu Val Phe Asp Asn
      35                  40                  45

gat gga aaa cct cgt gtg gat atc tta aag gcg cat ctt atg aag gag   309
Asp Gly Lys Pro Arg Val Asp Ile Leu Lys Ala His Leu Met Lys Glu
  50                  55                  60                  65

gga agg ctg gaa gag agt gtt gca ttg aga ata ata aca gag ggt gca   357
Gly Arg Leu Glu Glu Ser Val Ala Leu Arg Ile Ile Thr Glu Gly Ala
                  70                  75                  80

tca att ctt cga cag gaa aaa aat ttg ctg gat att gat gcg cca gtc   405
Ser Ile Leu Arg Gln Glu Lys Asn Leu Leu Asp Ile Asp Ala Pro Val
               85                  90                  95

act gtt tgt ggg gac att cat gga caa ttc ttt gat ttg atg aag ctc   453
Thr Val Cys Gly Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu
          100                 105                 110

ttt gaa gtc ggg gga tct cct gcc aac act cgc tac ctc ttc tta ggg   501
Phe Glu Val Gly Gly Ser Pro Ala Asn Thr Arg Tyr Leu Phe Leu Gly
      115                 120                 125

gac tat gtt gac aga ggg tac ttc agt att gaa tgt gtg ctg tat ttg   549
Asp Tyr Val Asp Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu
  130                 135                 140                 145

tgg gcc ttg aaa att ctc tac ccc aaa aca ctg ttt tta ctt cgt gga   597
Trp Ala Leu Lys Ile Leu Tyr Pro Lys Thr Leu Phe Leu Leu Arg Gly
                  150                 155                 160

aat cat gaa tgt aga cat cta aca gag tat ttc aca ttt aaa caa gaa   645
Asn His Glu Cys Arg His Leu Thr Glu Tyr Phe Thr Phe Lys Gln Glu
               165                 170                 175

tgt aaa ata aag tat tca gaa cga gta tat gat gcc tgt atg gat gcc   693
Cys Lys Ile Lys Tyr Ser Glu Arg Val Tyr Asp Ala Cys Met Asp Ala
          180                 185                 190

ttt gac tgc ctt ccc ctg gct gcc ctg atg aac caa cag ttc ctg tgt   741
Phe Asp Cys Leu Pro Leu Ala Ala Leu Met Asn Gln Gln Phe Leu Cys
      195                 200                 205

gtg cat ggt ggt ttg tct cca gag att aac act tta gat gat atc aga   789
Val His Gly Gly Leu Ser Pro Glu Ile Asn Thr Leu Asp Asp Ile Arg
  210                 215                 220                 225

aaa tta gac cga ttc aaa gaa cca cct gca tat gga cct atg tgt gat   837
Lys Leu Asp Arg Phe Lys Glu Pro Pro Ala Tyr Gly Pro Met Cys Asp
                  230                 235                 240
```

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atc ctg tgg tca gac ccc ctg gaa gat ttt gga aat gag aag act cag   885
Ile Leu Trp Ser Asp Pro Leu Glu Asp Phe Gly Asn Glu Lys Thr Gln
            245                 250                 255

gaa cat ttc act cac aac aca gtc agg ggg tgt tca tac ttc tac agt   933
Glu His Phe Thr His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser
        260                 265                 270

tac ccg gct gta tgt gaa ttc tta cag cac aat aac ttg tta tct ata   981
Tyr Pro Ala Val Cys Glu Phe Leu Gln His Asn Asn Leu Leu Ser Ile
    275                 280                 285

ctc cga gcc cac gaa gcc caa gat gca ggg tac cgc atg tac agg aaa   1029
Leu Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys
290                 295                 300                 305

agc caa aca aca ggc ttc cct tct cta att aca att ttt tca gca cca   1077
Ser Gln Thr Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro
                310                 315                 320

aat tac tta gat gta tac aat aac aaa gct gca gta ttg aag tat gag   1125
Asn Tyr Leu Asp Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu
            325                 330                 335

aac aat gtt atg aat atc agg caa ttc aac tgt tct cct cat cca tac   1173
Asn Asn Val Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr
        340                 345                 350

tgg ctt cca aat ttc atg gat gtt ttt act tgg tcc ctt cca ttt gtt   1221
Trp Leu Pro Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val
    355                 360                 365

ggg gaa aaa gtg act gag atg ctg gta aat gtc ctc aac atc tgc tca   1269
Gly Glu Lys Val Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser
370                 375                 380                 385

gat gat gaa cta ggg tca gaa gaa gat gga ttt gat ggt gca aca gct   1317
Asp Asp Glu Leu Gly Ser Glu Glu Asp Gly Phe Asp Gly Ala Thr Ala
                390                 395                 400

gca gcc cgg aaa gag gtg ata agg aac aag atc cga gca ata ggc aaa   1365
Ala Ala Arg Lys Glu Val Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys
            405                 410                 415

atg gcc aga gtg ttc tca gtg ctc aga gaa gag agt gag agt gtg ctg   1413
Met Ala Arg Val Phe Ser Val Leu Arg Glu Glu Ser Glu Ser Val Leu
        420                 425                 430

acg ctg aaa ggc ttg acc cca act ggc atg ctc ccc agc gga gta ctt   1461
Thr Leu Lys Gly Leu Thr Pro Thr Gly Met Leu Pro Ser Gly Val Leu
    435                 440                 445

tct gga ggg aag caa acc ctg caa agc gct atc aaa gga ttt tca cca   1509
Ser Gly Gly Lys Gln Thr Leu Gln Ser Ala Ile Lys Gly Phe Ser Pro
450                 455                 460                 465

caa cat aag atc act agc ttc gag gaa gcc aag ggc tta gac cga att   1557
Gln His Lys Ile Thr Ser Phe Glu Glu Ala Lys Gly Leu Asp Arg Ile
                470                 475                 480

aat gag agg atg ccg cct cgc aga gat gcc atg ccc tct gac gcc aac   1605
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Asn Glu Arg Met Pro Pro Arg Arg Asp Ala Met Pro Ser Asp Ala Asn
            485                 490                 495

ctt aac tcc atc aac aag gct ctc acc tca gag act aac ggc acg gac   1653
Leu Asn Ser Ile Asn Lys Ala Leu Thr Ser Glu Thr Asn Gly Thr Asp
        500                 505                 510

agc aat ggc agt aat agc agc aat att cag tga ttaactaggg taccccgggg 1706
Ser Asn Gly Ser Asn Ser Ser Asn Ile Gln
    515                 520

taccaaagag gagaaattaa ct atg gga aat gag gca agt tat cct ttg gaa  1758
                       Met Gly Asn Glu Ala Ser Tyr Pro Leu Glu
                       525                 530

atg tgc tca cac ttt gat gca gat gaa att aaa agg cta gga aag aga   1806
Met Cys Ser His Phe Asp Ala Asp Glu Ile Lys Arg Leu Gly Lys Arg
535                 540                 545                 550

ttt aag aag ctc gat ttg gac aat tct ggt tct ttg agt gtg gaa gag   1854
Phe Lys Lys Leu Asp Leu Asp Asn Ser Gly Ser Leu Ser Val Glu Glu
                555                 560                 565

ttc atg tct cta cct gag tta caa cag aat ccc tta gta cag cga gta   1902
Phe Met Ser Leu Pro Glu Leu Gln Gln Asn Pro Leu Val Gln Arg Val
            570                 575                 580

ata gat ata ttc gac aca gat ggg aat gga gaa gta gac ttt aaa gag   1950
Ile Asp Ile Phe Asp Thr Asp Gly Asn Gly Glu Val Asp Phe Lys Glu
        585                 590                 595

ttc att gag gga gtc tct cag ttc agt gtc aaa gga gat aag gaa cag   1998
Phe Ile Glu Gly Val Ser Gln Phe Ser Val Lys Gly Asp Lys Glu Gln
    600                 605                 610

aag ttg agg ttt gct ttc cgt atc tat gac atg gat aaa gac ggc tat   2046
Lys Leu Arg Phe Ala Phe Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr
615                 620                 625                 630

att tcc aat ggg gaa ctc ttc cag gtg cta aag atg atg gtg ggg aac   2094
Ile Ser Asn Gly Glu Leu Phe Gln Val Leu Lys Met Met Val Gly Asn
                635                 640                 645

aat ctg aaa gat aca cag tta cag caa att gta gac aaa acc ata ata   2142
Asn Leu Lys Asp Thr Gln Leu Gln Gln Ile Val Asp Lys Thr Ile Ile
            650                 655                 660

aat gca gat aag gat ggg gat gga aga ata tcc ttt gaa gaa ttc tgt   2190
Asn Ala Asp Lys Asp Gly Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys
        665                 670                 675

gct gtt gta ggc ggc cta gat atc cac aaa aag atg gtg gta gat gtg   2238
Ala Val Val Gly Gly Leu Asp Ile His Lys Lys Met Val Val Asp Val
    680                 685                 690

tga ttaattagaa gcttaattag ctgagcttgg actcctgttg atagatccag        2291

695

taatgacctc agaactccat ctggatttgt tcagaacgct cggttgccgc cgggcgtttt 2351

ttattggtga gaatccaagc tagcttggcg agattttcag gagctaagga agctaaaatg 2411
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gagaaaaaaa tcactggata taccaccgtt gatatatccc aatggcatcg taaagaacat 2471

tttgaggcat ttcagtcagt tgctcaatgt acctataacc agaccgttca gctggatatt 2531

acggcctttt taaagaccgt aaagaaaaat aagcacaagt tttatccggc ctttattcac 2591

attcttgccc gcctgatgaa tgctcatccg gaatttcgta tggcaatgaa agacggtgag 2651

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caagatgtgg cgtgttacgg tgaaaacctg gcctatttcc ctaaagggtt tattgagaat 2831

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ggcagaatgc ttaatgaatt acaacagtac tgcgatgagt ggcagggcgg ggcgtaattt 3071

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Lys Ala Ile Asp Pro Lys Leu Ser Thr Thr Asp Arg Val Val Lys Ala
            20                  25                  30

Val Pro Phe Pro Pro Ser His Arg Leu Thr Ala Lys Glu Val Phe Asp
        35                  40                  45

Asn Asp Gly Lys Pro Arg Val Asp Ile Leu Lys Ala His Leu Met Lys
    50                  55                  60
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Glu Gly Arg Leu Glu Glu Ser Val Ala Leu Arg Ile Ile Thr Glu Gly
 65                  70                  75                  80

Ala Ser Ile Leu Arg Gln Glu Lys Asn Leu Leu Asp Ile Asp Ala Pro
                 85                  90                  95

Val Thr Val Cys Gly Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys
            100                 105                 110

Leu Phe Glu Val Gly Gly Ser Pro Ala Asn Thr Arg Tyr Leu Phe Leu
        115                 120                 125

Gly Asp Tyr Val Asp Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr
    130                 135                 140

Leu Trp Ala Leu Lys Ile Leu Tyr Pro Lys Thr Leu Phe Leu Leu Arg
145                 150                 155                 160

Gly Asn His Glu Cys Arg His Leu Thr Glu Tyr Phe Thr Phe Lys Gln
                165                 170                 175

Glu Cys Lys Ile Lys Tyr Ser Glu Arg Val Tyr Asp Ala Cys Met Asp
            180                 185                 190

Ala Phe Asp Cys Leu Pro Leu Ala Ala Leu Met Asn Gln Gln Phe Leu
        195                 200                 205

Cys Val His Gly Gly Leu Ser Pro Glu Ile Asn Thr Leu Asp Asp Ile
    210                 215                 220

Arg Lys Leu Asp Arg Phe Lys Glu Pro Pro Ala Tyr Gly Pro Met Cys
225                 230                 235                 240

Asp Ile Leu Trp Ser Asp Pro Leu Glu Asp Phe Gly Asn Glu Lys Thr
                245                 250                 255

Gln Glu His Phe Thr His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr
            260                 265                 270

Ser Tyr Pro Ala Val Cys Glu Phe Leu Gln His Asn Asn Leu Leu Ser
        275                 280                 285

Ile Leu Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg
    290                 295                 300

Lys Ser Gln Thr Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala
305                 310                 315                 320

Pro Asn Tyr Leu Asp Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr
                325                 330                 335

Glu Asn Asn Val Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro
            340                 345                 350

Tyr Trp Leu Pro Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe
        355                 360                 365

Val Gly Glu Lys Val Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys
    370                 375                 380

Ser Asp Asp Glu Leu Gly Ser Glu Glu Asp Gly Phe Asp Gly Ala Thr
385                 390                 395                 400
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Ala Ala Ala Arg Lys Glu Val Ile Arg Asn Lys Ile Arg Ala Ile Gly
405 410 415

Lys Met Ala Arg Val Phe Ser Val Leu Arg Glu Glu Ser Glu Ser Val
420 425 430

Leu Thr Leu Lys Gly Leu Thr Pro Thr Gly Met Leu Pro Ser Gly Val
435 440 445

Leu Ser Gly Gly Lys Gln Thr Leu Gln Ser Ala Ile Lys Gly Phe Ser
450 455 460

Pro Gln His Lys Ile Thr Ser Phe Glu Glu Ala Lys Gly Leu Asp Arg
465 470 475 480

Ile Asn Glu Arg Met Pro Pro Arg Arg Asp Ala Met Pro Ser Asp Ala
485 490 495

Asn Leu Asn Ser Ile Asn Lys Ala Leu Thr Ser Glu Thr Asn Gly Thr
500 505 510

Asp Ser Asn Gly Ser Asn Ser Ser Asn Ile Gln
515 520

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20 25 30

Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Leu Pro Glu
35 40 45

Leu Gln Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
50 55 60

Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val Ser
65 70 75 80

Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
85 90 95

Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
100 105 110

Phe Gln Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
115 120 125

Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Gly
130 135 140

Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly Leu
145 150 155 160

Asp Ile His Lys Lys Met Val Val Asp Val
165 170

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<223> ribosomal binding site, multiple cloning site 2

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Met
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aga gga tcg cat cac cat cac cat cac gga tcc gcc gcc ccg gag ccg 165
Arg Gly Ser His His His His His His Gly Ser Ala Ala Pro Glu Pro
5 10 15

gcc cgg gct gca ccg ccc cca ccc ccg ccc ccg ccg ccc cct ccc ggg 213
Ala Arg Ala Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Gly
20 25 30

gct gac cgc gtc gtc aaa gct gtc cct ttc ccc cca aca cat cgc ttg 261
Ala Asp Arg Val Val Lys Ala Val Pro Phe Pro Pro Thr His Arg Leu
35 40 45

aca tct gaa gaa gta ttt gat ttg gat ggg ata ccc agg gtt gat gtt 309
Thr Ser Glu Glu Val Phe Asp Leu Asp Gly Ile Pro Arg Val Asp Val
50 55 60 65

ctg aag aac cac ttg gtg aaa gaa ggt cga gta gat gaa gaa att gcg 357
Leu Lys Asn His Leu Val Lys Glu Gly Arg Val Asp Glu Glu Ile Ala
70 75 80

ctt aga att atc aat gag ggt gct gcc atc ctt cgg aga gag aaa acc 405
Leu Arg Ile Ile Asn Glu Gly Ala Ala Ile Leu Arg Arg Glu Lys Thr
85 90 95

atg ata gaa gta gaa gct cca atc aca gtg tgt ggt gac atc cat ggc 453
Met Ile Glu Val Glu Ala Pro Ile Thr Val Cys Gly Asp Ile His Gly
100 105 110

caa ttt ttt gat ctg atg aaa ctt ttt gaa gta gga gga tca cct gct 501
Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser Pro Ala

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aat aca cga tac ctt ttt ctt ggc gat tat gtg gac aga ggt tat ttt   549
Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly Tyr Phe
130                 135                 140                 145

agt ata gag tgt gtc tta tat tta tgg gtt ctg aag att cta tac cca   597
Ser Ile Glu Cys Val Leu Tyr Leu Trp Val Leu Lys Ile Leu Tyr Pro
                150                 155                 160

agc aca tta ttt ctt ctg aga ggc aac cat gaa tgc aga cac ctt act   645
Ser Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg His Leu Thr
            165                 170                 175

gaa tat ttt acc ttt aag cag gaa tgt aaa att aag tat tcg gaa aga   693
Glu Tyr Phe Thr Phe Lys Gln Glu Cys Lys Ile Lys Tyr Ser Glu Arg
        180                 185                 190

gtc tat gaa gct tgt atg gaa gct ttt gat agt ttg cct ctt gct gca   741
Val Tyr Glu Ala Cys Met Glu Ala Phe Asp Ser Leu Pro Leu Ala Ala
    195                 200                 205

ctt tta aac caa cag ttt ctt tgt gtt cat ggt gga ctt tca cca gaa   789
Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly Leu Ser Pro Glu
210                 215                 220                 225

ata cac aca ctg gat gat att agg aga tta gat aga ttc aaa gag cca   837
Ile His Thr Leu Asp Asp Ile Arg Arg Leu Asp Arg Phe Lys Glu Pro
                230                 235                 240

cct gca ttt gga cca atg tgt gac ttg tta tgg tcc gat cct tct gaa   885
Pro Ala Phe Gly Pro Met Cys Asp Leu Leu Trp Ser Asp Pro Ser Glu
            245                 250                 255

gat ttt gga aat gaa aaa tca cag gaa cat ttt agt cac aat aca gtt   933
Asp Phe Gly Asn Glu Lys Ser Gln Glu His Phe Ser His Asn Thr Val
        260                 265                 270

cga gga tgt tct tat ttt tat aac tat cca gca gtg tgt gaa ttt ttg   981
Arg Gly Cys Ser Tyr Phe Tyr Asn Tyr Pro Ala Val Cys Glu Phe Leu
    275                 280                 285

caa aac aat aat ttg tta tcg att att aga gct cat gaa gct caa gat   1029
Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp
290                 295                 300                 305

gca ggc tat aga atg tac aga aaa agt caa act aca ggg ttc cct tca   1077
Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Thr Thr Gly Phe Pro Ser
                310                 315                 320

tta ata aca att ttt tcg gca cct aat tac tta gat gtc tac aat aat   1125
Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr Asn Asn
            325                 330                 335

aaa gct gct gta tta aag tat gaa aat aat gtg atg aat att cga cag   1173
Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn Ile Arg Gln
        340                 345                 350

ttt aac tgt tct cca cat cct tac tgg ttg cct aat ttt atg gat gtc   1221
Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe Met Asp Val
    355                 360                 365
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ttc acg tgg tct tta ccg ttt gtt gga gaa aaa gtg aca gaa atg ttg   1269
Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu Met Leu
370                 375                 380                 385

gta aat gtt ctg agt att tgc tct gat gat gaa cta atg act gaa ggt   1317
Val Asn Val Leu Ser Ile Cys Ser Asp Asp Glu Leu Met Thr Glu Gly
                390                 395                 400

gaa gac cag ttt gat ggt tca gct gca gcc cgg aaa gaa atc ata aga   1365
Glu Asp Gln Phe Asp Gly Ser Ala Ala Ala Arg Lys Glu Ile Ile Arg
            405                 410                 415

aac aaa att cga gca att ggc aag atg gca aga gtc ttc tct gtt ctc   1413
Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser Val Leu
        420                 425                 430

agg gag gag agt gaa agt gtg ctg aca ctc aag ggc ctg act ccc aca   1461
Arg Glu Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr Pro Thr
    435                 440                 445

ggg atg ttg cct agt gga gtg tta gct gga gga cgg cag acc ctg caa   1509
Gly Met Leu Pro Ser Gly Val Leu Ala Gly Gly Arg Gln Thr Leu Gln
450                 455                 460                 465

agt gcc aca gtt gag gct att gag gct gaa aaa gca ata cga gga ttc   1557
Ser Ala Thr Val Glu Ala Ile Glu Ala Glu Lys Ala Ile Arg Gly Phe
                470                 475                 480

tct cca cca cat aga atc tgc agt ttt gaa gag gca aag ggt ttg gat   1605
Ser Pro Pro His Arg Ile Cys Ser Phe Glu Glu Ala Lys Gly Leu Asp
            485                 490                 495

agg atc aat gag aga atg cca cct cgg aaa gat gct gta cag caa gat   1653
Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ala Val Gln Gln Asp
        500                 505                 510

ggt ttc aat tct ctg aac acc gca cat gcc act gag aac cac ggg acg   1701
Gly Phe Asn Ser Leu Asn Thr Ala His Ala Thr Glu Asn His Gly Thr
    515                 520                 525

ggc aac cat act gcc cag tga ttaactaggg taccccgggg aaagaggaga     1752
Gly Asn His Thr Ala Gln
530                 535

aattaact atg gga aat gag gca agt tat cct ttg gaa atg tgc tca cac  1802
          Met Gly Asn Glu Ala Ser Tyr Pro Leu Glu Met Cys Ser His
                    540                 545                 550

ttt gat gcr gat gaa att aaa agg cta gga aag aga ttt aag aag cty   1850
Phe Asp Xaa Asp Glu Ile Lys Arg Leu Gly Lys Arg Phe Lys Lys Xaa
                555                 560                 565

gat ttg gac aat tct ggt tct ttg agt gtg gaa gag ttc atg tct ctr   1898
Asp Leu Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Xaa
            570                 575                 580

cct gag tta caa cag aat ccy tta gta cag cga gta ata gat ata ttc   1946
Pro Glu Leu Gln Gln Asn Xaa Leu Val Gln Arg Val Ile Asp Ile Phe
        585                 590                 595

gac aca gat ggg aat gga gaa gta gac ttt aaa gar ttc att gag ggm   1994
Asp Thr Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Xaa
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     600                 605                 610

gtc tct cag ttc agt gtc aaa gga gat aag gar cag aar ttg agg ttt   2042
Val Ser Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe
615                 620                 625                 630

gct ttc cgt atc tat gac atg gat aaa gay ggc tat att tcc aat ggg   2090
Ala Phe Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly
                635                 640                 645

gaa ctc ttc cag gtr ytr aag atg atg gtg ggg aac aat ctg aaa gat   2138
Glu Leu Phe Gln Xaa Xaa Lys Met Met Val Gly Asn Asn Leu Lys Asp
            650                 655                 660

aca cag tta cag caa att gta gac aaa acc ata ata aat gca gat aag   2186
Thr Gln Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys
        665                 670                 675

gat ggr gat gga aga ata tcc ttt gaa gaa ttc tgt gct gtt gta ggy   2234
Asp Xaa Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Xaa
    680                 685                 690

ggc cta gat atc cac aaa aag atg gtg gta gat gtg tga ttaattagaa    2283
Gly Leu Asp Ile His Lys Lys Met Val Val Asp Val
695                 700                 705

gcttaattag ctgagcttgg actcctgttg atagatccag taatgacctc agaactccat 2343

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gagcggatac atatttgaat gtatttagaa aaataaacaa atagggggttc cgcgcacatt 5463

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Pro Ala Arg Ala Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro
             20                  25                  30

Gly Ala Asp Arg Val Val Lys Ala Val Pro Phe Pro Pro Thr His Arg
         35                  40                  45

Leu Thr Ser Glu Glu Val Phe Asp Leu Asp Gly Ile Pro Arg Val Asp
     50                  55                  60

Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Val Asp Glu Glu Ile
 65                  70                  75                  80

Ala Leu Arg Ile Ile Asn Glu Gly Ala Ala Ile Leu Arg Arg Glu Lys
                 85                  90                  95

Thr Met Ile Glu Val Glu Ala Pro Ile Thr Val Cys Gly Asp Ile His
            100                 105                 110

Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser Pro
        115                 120                 125

Ala Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly Tyr
    130                 135                 140

Phe Ser Ile Glu Cys Val Leu Tyr Leu Trp Val Leu Lys Ile Leu Tyr
145                 150                 155                 160

Pro Ser Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg His Leu
                165                 170                 175

Thr Glu Tyr Phe Thr Phe Lys Gln Glu Cys Lys Ile Lys Tyr Ser Glu
            180                 185                 190

Arg Val Tyr Glu Ala Cys Met Glu Ala Phe Asp Ser Leu Pro Leu Ala
        195                 200                 205

Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly Leu Ser Pro
    210                 215                 220

Glu Ile His Thr Leu Asp Asp Ile Arg Arg Leu Asp Arg Phe Lys Glu
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225 230 235 240

Pro Pro Ala Phe Gly Pro Met Cys Asp Leu Leu Trp Ser Asp Pro Ser
245 250 255

Glu Asp Phe Gly Asn Glu Lys Ser Gln Glu His Phe Ser His Asn Thr
260 265 270

Val Arg Gly Cys Ser Tyr Phe Tyr Asn Tyr Pro Ala Val Cys Glu Phe
275 280 285

Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln
290 295 300

Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Thr Thr Gly Phe Pro
305 310 315 320

Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr Asn
325 330 335

Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn Ile Arg
340 345 350

Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe Met Asp
355 360 365

Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu Met
370 375 380

Leu Val Asn Val Leu Ser Ile Cys Ser Asp Asp Glu Leu Met Thr Glu
385 390 395 400

Gly Glu Asp Gln Phe Asp Gly Ser Ala Ala Ala Arg Lys Glu Ile Ile
405 410 415

Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser Val
420 425 430

Leu Arg Glu Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr Pro
435 440 445

Thr Gly Met Leu Pro Ser Gly Val Leu Ala Gly Gly Arg Gln Thr Leu
450 455 460

Gln Ser Ala Thr Val Glu Ala Ile Glu Ala Glu Lys Ala Ile Arg Gly
465 470 475 480

Phe Ser Pro Pro His Arg Ile Cys Ser Phe Glu Glu Ala Lys Gly Leu
485 490 495

Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ala Val Gln Gln
500 505 510

Asp Gly Phe Asn Ser Leu Asn Thr Ala His Ala Thr Glu Asn His Gly
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Thr Gly Asn His Thr Ala Gln
530 535

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20 25 30

Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Xaa Pro Glu
35 40 45

Leu Gln Gln Asn Xaa Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
50 55 60

Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Xaa Val Ser
65 70 75 80

Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
85 90 95

Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
100 105 110

Phe Gln Xaa Xaa Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
115 120 125

Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Xaa
130 135 140

Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Xaa Gly Leu
145 150 155 160

Asp Ile His Lys Lys Met Val Val Asp Val
165 170

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Arg Gly Ser His His His His His His Gly Ser Ala Ala Pro Glu Pro
                5                   10                  15

gcc cgg gct gca ccg ccc cca ccc ccg ccc ccg ccg ccc cct ccc ggg    213
Ala Arg Ala Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Gly
          20                  25                  30

gct gac cgc gtc gtc aaa gct gtc cct ttc ccc cca aca cat cgc ttg    261
Ala Asp Arg Val Val Lys Ala Val Pro Phe Pro Pro Thr His Arg Leu
      35                  40                  45

aca tct gaa gaa gta ttt gat ttg gat ggg ata ccc agg gtt gat gtt    309
Thr Ser Glu Glu Val Phe Asp Leu Asp Gly Ile Pro Arg Val Asp Val
 50                  55                  60                  65

ctg aag aac cac ttg gtg aaa gaa ggt cga gta gat gaa gaa att gcg    357
Leu Lys Asn His Leu Val Lys Glu Gly Arg Val Asp Glu Glu Ile Ala
                70                  75                  80

ctt aga att atc aat gag ggt gct gcc atc ctt cgg aga gag aaa acc    405
Leu Arg Ile Ile Asn Glu Gly Ala Ala Ile Leu Arg Arg Glu Lys Thr
            85                  90                  95

atg ata gaa gta gaa gct cca atc aca gtg tgt ggt gac atc cat ggc    453
Met Ile Glu Val Glu Ala Pro Ile Thr Val Cys Gly Asp Ile His Gly
        100                 105                 110

caa ttt ttt gat ctg atg aaa ctt ttt gaa gta gga gga tca cct gct    501
Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser Pro Ala
    115                 120                 125

aat aca cga tac ctt ttt ctt ggc gat tat gtg gac aga ggt tat ttt    549
Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly Tyr Phe
130                 135                 140                 145

agt ata gag cat gtt cta ggc act gaa gac ata tcg att aat cct cac    597
Ser Ile Glu His Val Leu Gly Thr Glu Asp Ile Ser Ile Asn Pro His
                150                 155                 160

aat aat att aat gag tgt gtc tta tat tta tgg gtt ctg aag att cta    645
Asn Asn Ile Asn Glu Cys Val Leu Tyr Leu Trp Val Leu Lys Ile Leu
            165                 170                 175

tac cca agc aca tta ttt ctt ctg aga ggc aac cat gaa tgc aga cac    693
Tyr Pro Ser Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg His
        180                 185                 190

ctt act gaa tat ttt acc ttt aag cag gaa tgt aaa att aag tat tcg    741
Leu Thr Glu Tyr Phe Thr Phe Lys Gln Glu Cys Lys Ile Lys Tyr Ser
    195                 200                 205

gaa aga gtc tat gaa gct tgt atg gaa gct ttt gat agt ttg cct ctt    789
Glu Arg Val Tyr Glu Ala Cys Met Glu Ala Phe Asp Ser Leu Pro Leu
210                 215                 220                 225

gct gca ctt tta aac caa cag ttt ctt tgt gtt cat ggt gga ctt tca    837
Ala Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly Leu Ser
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                    230                 235                 240

cca gaa ata cac aca ctg gat gat att agg aga tta gat aga ttc aaa   885
Pro Glu Ile His Thr Leu Asp Asp Ile Arg Arg Leu Asp Arg Phe Lys
            245                 250                 255

gag cca cct gca ttt gga cca atg tgt gac ttg tta tgg tcc gat cct   933
Glu Pro Pro Ala Phe Gly Pro Met Cys Asp Leu Leu Trp Ser Asp Pro
        260                 265                 270

tct gaa gat ttt gga aat gaa aaa tca cag gaa cat ttt agt cac aat   981
Ser Glu Asp Phe Gly Asn Glu Lys Ser Gln Glu His Phe Ser His Asn
    275                 280                 285

aca gtt cga gga tgt tct tat ttt tat aac tat cca gca gtg tgt gaa   1029
Thr Val Arg Gly Cys Ser Tyr Phe Tyr Asn Tyr Pro Ala Val Cys Glu
290                 295                 300                 305

ttt ttg caa aac aat aat ttg tta tcg att att aga gct cat gaa gct   1077
Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His Glu Ala
                310                 315                 320

caa gat gca ggc tat aga atg tac aga aaa agt caa act aca ggg ttc   1125
Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Thr Thr Gly Phe
            325                 330                 335

cct tca tta ata aca att ttt tcg gca cct aat tac tta gat gtc tac   1173
Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val Tyr
        340                 345                 350

aat aat aaa gct gct gta tta aag tat gaa aat aat gtg atg aat att   1221
Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn Ile
    355                 360                 365

cga cag ttt aac tgt tct cca cat cct tac tgg ttg cct aat ttt atg   1269
Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe Met
370                 375                 380                 385

gat gtc ttc acg tgg tct tta ccg ttt gtt gga gaa aaa gtg aca gaa   1317
Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu
                390                 395                 400

atg ttg gta aat gtt ctg agt att tgc tct gat gat gaa cta atg act   1365
Met Leu Val Asn Val Leu Ser Ile Cys Ser Asp Asp Glu Leu Met Thr
            405                 410                 415

gaa ggt gaa gac cag ttt gat ggt tca gct gca gcc cgg aaa gaa atc   1413
Glu Gly Glu Asp Gln Phe Asp Gly Ser Ala Ala Ala Arg Lys Glu Ile
        420                 425                 430

ata aga aac aaa att cga gca att ggc aag atg gca aga gtc ttc tct   1461
Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser
    435                 440                 445

gtt ctc agg gag gag agt gaa agt gtg ctg aca ctc aag ggc ctg act   1509
Val Leu Arg Glu Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr
450                 455                 460                 465

ccc aca ggg atg ttg cct agt gga gtg tta gct gga gga cgg cag acc   1557
Pro Thr Gly Met Leu Pro Ser Gly Val Leu Ala Gly Gly Arg Gln Thr
                470                 475                 480
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ctg caa agt gcc aca gtt gag gct att gag gct gaa aaa gca ata cga   1605
Leu Gln Ser Ala Thr Val Glu Ala Ile Glu Ala Glu Lys Ala Ile Arg
            485                 490                 495

gga ttc tct cca cca cat aga atc tgc agt ttt gaa gag gca aag ggt   1653
Gly Phe Ser Pro Pro His Arg Ile Cys Ser Phe Glu Glu Ala Lys Gly
        500                 505                 510

ttg gat agg atc aat gag aga atg cca cct cgg aaa gat gct gta cag   1701
Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ala Val Gln
    515                 520                 525

caa gat ggt ttc aat tct ctg aac acc gca cat gcc act gag aac cac   1749
Gln Asp Gly Phe Asn Ser Leu Asn Thr Ala His Ala Thr Glu Asn His
530                 535                 540                 545

ggg acg ggc aac cat act gcc cag tga ttaactaggg tacccc gggg       1796
Gly Thr Gly Asn His Thr Ala Gln
                550

aaagaggaga aattaact atg gga aat gag gca agt tat cct ttg gaa atg   1847
                    Met Gly Asn Glu Ala Ser Tyr Pro Leu Glu Met
                    555                 560                 565

tgc tca cac ttt gat gcr gat gaa att aaa agg cta gga aag aga ttt   1895
Cys Ser His Phe Asp Xaa Asp Glu Ile Lys Arg Leu Gly Lys Arg Phe
                570                 575                 580

aag aag cty gat ttg gac aat tct ggt tct ttg agt gtg gaa gag ttc   1943
Lys Lys Xaa Asp Leu Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe
            585                 590                 595

atg tct ctr cct gag tta caa cag aat ccy tta gta cag cga gta ata   1991
Met Ser Xaa Pro Glu Leu Gln Gln Asn Xaa Leu Val Gln Arg Val Ile
        600                 605                 610

gat ata ttc gac aca gat ggg aat gga gaa gta gac ttt aaa gar ttc   2039
Asp Ile Phe Asp Thr Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe
    615                 620                 625

att gag ggm gtc tct cag ttc agt gtc aaa gga gat aag gar cag aar   2087
Ile Glu Xaa Val Ser Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys
630                 635                 640                 645

ttg agg ttt gct ttc cgt atc tat gac atg gat aaa gay ggc tat att   2135
Leu Arg Phe Ala Phe Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile
                650                 655                 660

tcc aat ggg gaa ctc ttc cag gtr ytr aag atg atg gtg ggg aac aat   2183
Ser Asn Gly Glu Leu Phe Gln Xaa Xaa Lys Met Met Val Gly Asn Asn
            665                 670                 675

ctg aaa gat aca cag tta cag caa att gta gac aaa acc ata ata aat   2231
Leu Lys Asp Thr Gln Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn
        680                 685                 690

gca gat aag gat ggr gat gga aga ata tcc ttt gaa gaa ttc tgt gct   2279
Ala Asp Lys Asp Xaa Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala
    695                 700                 705

gtt gta ggy ggc cta gat atc cac aaa aag atg gtg gta gat gtg tga   2327
Val Val Xaa Gly Leu Asp Ile His Lys Lys Met Val Val Asp Val
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710 715 720 725

ttaattagaa gcttaattag ctgagcttgg actcctgttg atagatccag taatgacctc 2387

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taaagaccgt aaagaaaaat aagcacaagt tttatccggc ctttattcac attcttgccc 2687

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<212> PRT
<213> Homo sapiens

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Pro	Ala	Arg	Ala	Ala	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro
		20					25					30			

Gly Ala Asp Arg Val Val Lys Ala Val Pro Phe Pro Pro Thr His Arg
35 40 45

Leu Thr Ser Glu Glu Val Phe Asp Leu Asp Gly Ile Pro Arg Val Asp
50 55 60

Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Val Asp Glu Glu Ile
65 70 75 80

Ala Leu Arg Ile Ile Asn Glu Gly Ala Ala Ile Leu Arg Arg Glu Lys
85 90 95

Thr Met Ile Glu Val Glu Ala Pro Ile Thr Val Cys Gly Asp Ile His
100 105 110

Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly Gly Ser Pro
115 120 125

Ala Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg Gly Tyr
130 135 140

Phe Ser Ile Glu His Val Leu Gly Thr Glu Asp Ile Ser Ile Asn Pro
145 150 155 160

His Asn Asn Ile Asn Glu Cys Val Leu Tyr Leu Trp Val Leu Lys Ile
165 170 175

Leu Tyr Pro Ser Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys Arg
180 185 190

His Leu Thr Glu Tyr Phe Thr Phe Lys Gln Glu Cys Lys Ile Lys Tyr
195 200 205

Ser Glu Arg Val Tyr Glu Ala Cys Met Glu Ala Phe Asp Ser Leu Pro
210 215 220

Leu Ala Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly Leu
225 230 235 240

Ser Pro Glu Ile His Thr Leu Asp Asp Ile Arg Arg Leu Asp Arg Phe
245 250 255

Lys Glu Pro Pro Ala Phe Gly Pro Met Cys Asp Leu Leu Trp Ser Asp
260 265 270

Pro Ser Glu Asp Phe Gly Asn Glu Lys Ser Gln Glu His Phe Ser His
275 280 285

Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Asn Tyr Pro Ala Val Cys
290 295 300

Glu Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His Glu
305 310 315 320

Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Thr Thr Gly
325 330 335

Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp Val
340 345 350

Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met Asn

355 360 365

Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn Phe
370 375 380

Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr
385 390 395 400

Glu Met Leu Val Asn Val Leu Ser Ile Cys Ser Asp Asp Glu Leu Met
405 410 415

Thr Glu Gly Glu Asp Gln Phe Asp Gly Ser Ala Ala Ala Arg Lys Glu
420 425 430

Ile Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe
435 440 445

Ser Val Leu Arg Glu Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu
450 455 460

Thr Pro Thr Gly Met Leu Pro Ser Gly Val Leu Ala Gly Gly Arg Gln
465 470 475 480

Thr Leu Gln Ser Ala Thr Val Glu Ala Ile Glu Ala Glu Lys Ala Ile
485 490 495

Arg Gly Phe Ser Pro Pro His Arg Ile Cys Ser Phe Glu Glu Ala Lys
500 505 510

Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ala Val
515 520 525

Gln Gln Asp Gly Phe Asn Ser Leu Asn Thr Ala His Ala Thr Glu Asn
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His Gly Thr Gly Asn His Thr Ala Gln
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20 25 30

Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Xaa Pro Glu
35 40 45

Leu Gln Gln Asn Xaa Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
50 55 60

Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Xaa Val Ser
65 70 75 80

Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
85 90 95

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Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
            100                 105                 110

Phe Gln Xaa Xaa Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
        115                 120                 125

Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Xaa
    130                 135                 140

Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Xaa Gly Leu
145                 150                 155                     160

Asp Ile His Lys Lys Met Val Val Asp Val
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                                                             Met
                                                              1

aga gga tcg cat cac cat cac cat cac gga tcc atg tcc ggg agg cgc    165
Arg Gly Ser His His His His His His Gly Ser Met Ser Gly Arg Arg
            5                   10                  15

ttc cac ctc tcc acc acc gac cgc gtc atc aaa gct gtc ccc ttt cct    213
Phe His Leu Ser Thr Thr Asp Arg Val Ile Lys Ala Val Pro Phe Pro
        20                  25                  30

cca acc caa cgg ctt act ttc aag gaa gta ttt gag aat ggg aaa cct    261
Pro Thr Gln Arg Leu Thr Phe Lys Glu Val Phe Glu Asn Gly Lys Pro
    35                  40                  45

aaa gtt gat gtt tta aaa aac cat ttg gta aag gaa gga cga ctg gaa    309
Lys Val Asp Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Leu Glu
 50                  55                  60                  65

gag gaa gta gcc tta aag ata atc aat gat ggg gct gcc atc ctg agg    357
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Glu Glu Val Ala Leu Lys Ile Ile Asn Asp Gly Ala Ala Ile Leu Arg
                70                  75                  80

caa gag aag act atg ata gaa gta gat gct cca atc aca gta tgt ggt   405
Gln Glu Lys Thr Met Ile Glu Val Asp Ala Pro Ile Thr Val Cys Gly
            85                  90                  95

gat att cat gga caa ttc ttt gac cta atg aag tta ttt gaa gtt gga   453
Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly
        100                 105                 110

gga tca cct agt aac aca cgc tac ctc ttt ctg ggt gac tat gtg gac   501
Gly Ser Pro Ser Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp
    115                 120                 125

aga ggc tat ttc agt ata gag tgt gtg ctg tat tta tgg agt tta aag   549
Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu Trp Ser Leu Lys
130                 135                 140                 145

att aat cat ccc aaa aca ttg ttt ctg ctt cgg gga aat cat gaa tgc   597
Ile Asn His Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys
                150                 155                 160

agg cat ctt aca gac tat ttc acc ttc aaa cag gaa tgt cga atc aaa   645
Arg His Leu Thr Asp Tyr Phe Thr Phe Lys Gln Glu Cys Arg Ile Lys
            165                 170                 175

tat tcg gaa cag gtg tat gat gcc tgt atg gag aca ttt gac tgt ctt   693
Tyr Ser Glu Gln Val Tyr Asp Ala Cys Met Glu Thr Phe Asp Cys Leu
        180                 185                 190

cct ctt gct gcc ctc tta aac cag cag ttt ctc tgt gta cat gga gga   741
Pro Leu Ala Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly
    195                 200                 205

atg tca cct gaa att act tct tta gat gac att agg aaa tta gac agg   789
Met Ser Pro Glu Ile Thr Ser Leu Asp Asp Ile Arg Lys Leu Asp Arg
210                 215                 220                 225

ttt acg gaa cct ccc gcc ttt gga cct gtg tgt gac ctg ctt tgg tct   837
Phe Thr Glu Pro Pro Ala Phe Gly Pro Val Cys Asp Leu Leu Trp Ser
                230                 235                 240

gat ccc tca gag gat tat ggc aat gag aag acc ttg gag cac tat acc   885
Asp Pro Ser Glu Asp Tyr Gly Asn Glu Lys Thr Leu Glu His Tyr Thr
            245                 250                 255

cac aac act gtc cga ggg tgc tct tat ttc tac agt tac cct gca gtt   933
His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala Val
        260                 265                 270

tgt gaa ttt ttg cag aac aat aat tta cta tca att atc aga gcc cat   981
Cys Glu Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His
    275                 280                 285

gaa gcc caa gat gct ggg tat cga atg tac agg aag agc caa gcc aca   1029
Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Ala Thr
290                 295                 300                 305

ggc ttt cca tca ctt att aca att ttc tct gcc ccc aat tac cta gat   1077
Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp
                310                 315                 320
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gtc tat aac aat aaa gct gct gtg ttg aaa tat gaa aac aat gtc atg   1125
Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met
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aat atc agg cag ttt aac tgt tct cca cac ccc tac tgg ctt cca aac   1173
Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn
        340                 345                 350

ttt atg gat gtt ttc aca tgg tct ttg cct ttt gtt ggg gaa aaa gtc   1221
Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val
    355                 360                 365

aca gag atg ctg gta aat gtg ctc aac ata tgc tct gat gac gaa ctg   1269
Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu Leu
370                 375                 380                 385

att tct gat gat gaa gca gaa gga agc act aca gtt cgt aag gag atc   1317
Ile Ser Asp Asp Glu Ala Glu Gly Ser Thr Thr Val Arg Lys Glu Ile
            390                 395                 400

atc agg aat aag atc aga gcc att ggg aag atg gca cgg gtc ttt tca   1365
Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser
            405                 410                 415

att ctt cgg caa gaa agt gag agt gtg ctg act ctc aag ggc ctg act   1413
Ile Leu Arg Gln Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr
        420                 425                 430

ccc aca ggc aca ctc cct ctg ggc gtc ctc tca gga ggc aag cag act   1461
Pro Thr Gly Thr Leu Pro Leu Gly Val Leu Ser Gly Gly Lys Gln Thr
    435                 440                 445

atc gag aca gcc atc aga ggg ttc tcg ctt cag cac aag atc cgg agt   1509
Ile Glu Thr Ala Ile Arg Gly Phe Ser Leu Gln His Lys Ile Arg Ser
450                 455                 460                 465

ttt gaa gaa gcg cga ggt ctg gac cga att aat gag cga atg cca ccc   1557
Phe Glu Glu Ala Arg Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro
                470                 475                 480

cga aag gat agc ata tac cct ggt ggg cca atg aaa tct gta acc tca   1605
Arg Lys Asp Ser Ile Tyr Pro Gly Gly Pro Met Lys Ser Val Thr Ser
            485                 490                 495

gca cac tca cat gct gcg cac agg agc gac caa ggg aag aaa gcc cat   1653
Ala His Ser His Ala Ala His Arg Ser Asp Gln Gly Lys Lys Ala His
        500                 505                 510

tca tga ttaactaggg taccccgggg taccaaagag gagaaattaa ct atg gga   1707
Ser                                                 Met Gly
    515

aat gag gca agt tat cct ttg gaa atg tgc tca cac ttt gat gca gat   1755
Asn Glu Ala Ser Tyr Pro Leu Glu Met Cys Ser His Phe Asp Ala Asp
        520                 525                 530

gaa att aaa agg cta gga aag aga ttt aag aag ctc gat ttg gac aat   1803
Glu Ile Lys Arg Leu Gly Lys Arg Phe Lys Lys Leu Asp Leu Asp Asn
    535                 540                 545

tct ggt tct ttg agt gtg gaa gag ttc atg tct cta cct gag tta caa   1851
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Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Leu Pro Glu Leu Gln
550                 555                 560                 565

cag aat ccc tta gta cag cga gta ata gat ata ttc gac aca gat ggg   1899
Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr Asp Gly
                570                 575                 580

aat gga gaa gta gac ttt aaa gag ttc att gag gga gtc tct cag ttc   1947
Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val Ser Gln Phe
            585                 590                 595

agt gtc aaa gga gat aag gaa cag aag ttg agg ttt gct ttc cgt atc   1995
Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe Arg Ile
        600                 605                 610

tat gac atg gat aaa gac ggc tat att tcc aat ggg gaa ctc ttc cag   2043
Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu Phe Gln
    615                 620                 625

gtg cta aag atg atg gtg ggg aac aat ctg aaa gat aca cag tta cag   2091
Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln Leu Gln
630                 635                 640                 645

caa att gta gac aaa acc ata ata aat gca gat aag gat ggg gat gga   2139
Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Gly Asp Gly
                650                 655                 660

aga ata tcc ttt gaa gaa ttc tgt gct gtt gta ggc ggc cta gat atc   2187
Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly Leu Asp Ile
            665                 670                 675

cac aaa aag atg gtg gta gat gtg tga ttaattagaa gcttaattag         2234
His Lys Lys Met Val Val Asp Val
        680                 685

ctgagcttgg actcctgttg atagatccag taatgacctc agaactccat ctggatttgt 2294

tcagaacgct cggttgccgc cgggcgtttt ttattggtga gaatccaagc tagcttggcg 2354

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gatatatccc aatggcatcg taaagaacat tttgaggcat ttcagtcagt tgctcaatgt 2474

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Pro Lys Val Asp Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Leu
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Glu Glu Glu Val Ala Leu Lys Ile Ile Asn Asp Gly Ala Ala Ile Leu
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Arg Gln Glu Lys Thr Met Ile Glu Val Asp Ala Pro Ile Thr Val Cys
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Gly Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val
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Gly Gly Ser Pro Ser Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val
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Asp Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu Trp Ser Leu
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Lys Ile Asn His Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu
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Cys Arg His Leu Thr Asp Tyr Phe Thr Phe Lys Gln Glu Cys Arg Ile
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Lys Tyr Ser Glu Gln Val Tyr Asp Ala Cys Met Glu Thr Phe Asp Cys
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Arg Phe Thr Glu Pro Pro Ala Phe Gly Pro Val Cys Asp Leu Leu Trp
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Ser Asp Pro Ser Glu Asp Tyr Gly Asn Glu Lys Thr Leu Glu His Tyr
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Thr His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala
            260                 265                 270

Val Cys Glu Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala
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His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Ala
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Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu
305                 310                 315                     320

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Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro
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Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys
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Val Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu
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Leu Ile Ser Asp Asp Glu Ala Glu Gly Ser Thr Thr Val Arg Lys Glu
385                 390                 395                     400

Ile Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe
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Ser Ile Leu Arg Gln Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu
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Thr Pro Thr Gly Thr Leu Pro Leu Gly Val Leu Ser Gly Gly Lys Gln
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Thr Ile Glu Thr Ala Ile Arg Gly Phe Ser Leu Gln His Lys Ile Arg
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Ser Phe Glu Glu Ala Arg Gly Leu Asp Arg Ile Asn Glu Arg Met Pro
465                 470                 475                     480

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35 40 45

Leu Gln Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
50 55 60

Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val Ser
65 70 75 80

Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
85 90 95

Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
100 105 110

Phe Gln Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
115 120 125

Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Gly
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Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly Leu
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Asp Ile His Lys Lys Met Val Val Asp Val
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Met
1

aga gga tcg cat cac cat cac cat cac gga tcc atg tcc ggg agg cgc 165
Arg Gly Ser His His His His His His Gly Ser Met Ser Gly Arg Arg
5 10 15

ttc cac ctc tcc acc acc gac cgc gtc atc aaa gct gtc ccc ttt cct 213
Phe His Leu Ser Thr Thr Asp Arg Val Ile Lys Ala Val Pro Phe Pro
20 25 30

cca acc caa cgg ctt act ttc aag gaa gta ttt gag aat ggg aaa cct 261
Pro Thr Gln Arg Leu Thr Phe Lys Glu Val Phe Glu Asn Gly Lys Pro
35 40 45

aaa gtt gat gtt tta aaa aac cat ttg gta aag gaa gga cga ctg gaa 309
Lys Val Asp Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Leu Glu
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Glu Glu Val Ala Leu Lys Ile Ile Asn Asp Gly Ala Ala Ile Leu Arg
70 75 80

caa gag aag act atg ata gaa gta gat gct cca atc aca gta tgt ggt 405
Gln Glu Lys Thr Met Ile Glu Val Asp Ala Pro Ile Thr Val Cys Gly
85 90 95

gat att cat gga caa ttc ttt gac cta atg aag tta ttt gaa gtt gga 453
Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val Gly
100 105 110

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Gly Ser Pro Ser Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val Asp
115 120 125

aga ggc tat ttc agt ata gag tgt gtg ctg tat tta tgg agt tta aag 549
Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu Trp Ser Leu Lys
130 135 140 145

att aat cat ccc aaa aca ttg ttt ctg ctt cgg gga aat cat gaa tgc 597
Ile Asn His Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu Cys
150 155 160

agg cat ctt aca gac tat ttc acc ttc aaa cag gaa tgt cga atc aaa 645
Arg His Leu Thr Asp Tyr Phe Thr Phe Lys Gln Glu Cys Arg Ile Lys
165 170 175

tat tcg gaa cag gtg tat gat gcc tgt atg gag aca ttt gac tgt ctt 693

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Tyr Ser Glu Gln Val Tyr Asp Ala Cys Met Glu Thr Phe Asp Cys Leu
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cct ctt gct gcc ctc tta aac cag cag ttt ctc tgt gta cat gga gga   741
Pro Leu Ala Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly Gly
    195                 200                 205

atg tca cct gaa att act tct tta gat gac att agg aaa tta gac agg   789
Met Ser Pro Glu Ile Thr Ser Leu Asp Asp Ile Arg Lys Leu Asp Arg
210                 215                 220                 225

ttt acg gaa cct ccc gcc ttt gga cct gtg tgt gac ctg ctt tgg tct   837
Phe Thr Glu Pro Pro Ala Phe Gly Pro Val Cys Asp Leu Leu Trp Ser
                230                 235                 240

gat ccc tca gag gat tat ggc aat gag aag acc ttg gag cac tat acc   885
Asp Pro Ser Glu Asp Tyr Gly Asn Glu Lys Thr Leu Glu His Tyr Thr
            245                 250                 255

cac aac act gtc cga ggg tgc tct tat ttc tac agt tac cct gca gtt   933
His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala Val
        260                 265                 270

tgt gaa ttt ttg cag aac aat aat tta cta tca att atc aga gcc cat   981
Cys Glu Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala His
    275                 280                 285

gaa gcc caa gat gct ggg tat cga atg tac agg aag agc caa gcc aca   1029
Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Ala Thr
290                 295                 300                 305

ggc ttt cca tca ctt att aca att ttc tct gcc ccc aat tac cta gat   1077
Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu Asp
                310                 315                 320

gtc tat aac aat aaa gct gct gtg ttg aaa tat gaa aac aat gtc atg   1125
Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val Met
            325                 330                 335

aat atc agg cag ttt aac tgt tct cca cac ccc tac tgg ctt cca aac   1173
Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro Asn
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ttt atg gat gtt ttc aca tgg tct ttg cct ttt gtt ggg gaa aaa gtc   1221
Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val
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aca gag atg ctg gta aat gtg ctc aac ata tgc tct gat gac gaa ctg   1269
Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu Leu
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att tct gat gat gaa gca gaa gga agc act aca gtt cgt aag gag atc   1317
Ile Ser Asp Asp Glu Ala Glu Gly Ser Thr Thr Val Arg Lys Glu Ile
                390                 395                 400

atc agg aat aag atc aga gcc att ggg aag atg gca cgg gtc ttt tca   1365
Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe Ser
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att ctt cgg caa gaa agt gag agt gtg ctg act ctc aag ggc ctg act   1413
Ile Leu Arg Gln Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu Thr
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ccc aca ggc aca ctc cct ctg ggc gtc ctc tca gga ggc aag cag act   1461
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atc gag aca gcc aaa caa gaa gcc gca gag gag cgg gaa gcc atc aga   1509
Ile Glu Thr Ala Lys Gln Glu Ala Ala Glu Glu Arg Glu Ala Ile Arg
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ctg gac cga att aat gag cga atg cca ccc cga aag gat agc ata tac   1605
Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ser Ile Tyr
            485                 490                 495

cct ggt ggg cca atg aaa tct gta acc tca gca cac tca cat gct gcg   1653
Pro Gly Gly Pro Met Lys Ser Val Thr Ser Ala His Ser His Ala Ala
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His Arg Ser Asp Gln Gly Lys Lys Ala His Ser
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Pro Leu Glu Met Cys Ser His Phe Asp Ala Asp Glu Ile Lys Arg Leu
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gga aag aga ttt aag aag ctc gat ttg gac aat tct ggt tct ttg agt   1848
Gly Lys Arg Phe Lys Lys Leu Asp Leu Asp Asn Ser Gly Ser Leu Ser
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gtg gaa gag ttc atg tct cta cct gag tta caa cag aat ccc tta gta   1896
Val Glu Glu Phe Met Ser Leu Pro Glu Leu Gln Gln Asn Pro Leu Val
565                 570                 575                 580

cag cga gta ata gat ata ttc gac aca gat ggg aat gga gaa gta gac   1944
Gln Arg Val Ile Asp Ile Phe Asp Thr Asp Gly Asn Gly Glu Val Asp
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ttt aaa gag ttc att gag gga gtc tct cag ttc agt gtc aaa gga gat   1992
Phe Lys Glu Phe Ile Glu Gly Val Ser Gln Phe Ser Val Lys Gly Asp
            600                 605                 610

aag gaa cag aag ttg agg ttt gct ttc cgt atc tat gac atg gat aaa   2040
Lys Glu Gln Lys Leu Arg Phe Ala Phe Arg Ile Tyr Asp Met Asp Lys
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gac ggc tat att tcc aat ggg gaa ctc ttc cag gtg cta aag atg atg   2088
Asp Gly Tyr Ile Ser Asn Gly Glu Leu Phe Gln Val Leu Lys Met Met
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gtg ggg aac aat ctg aaa gat aca cag tta cag caa att gta gac aaa   2136
Val Gly Asn Asn Leu Lys Asp Thr Gln Leu Gln Gln Ile Val Asp Lys
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Glu Phe Cys Ala Val Val Gly Gly Leu Asp Ile His Lys Lys Met Val
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Val Asp Val
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tcac 5528

<210> 33
<211> 524

<212> PRT
<213> Homo sapiens

<400> 33

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Met Arg Gly Ser His His His His His His Gly Ser Met Ser Gly Arg
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Arg Phe His Leu Ser Thr Thr Asp Arg Val Ile Lys Ala Val Pro Phe
             20                  25                  30

Pro Pro Thr Gln Arg Leu Thr Phe Lys Glu Val Phe Glu Asn Gly Lys
         35                  40                  45

Pro Lys Val Asp Val Leu Lys Asn His Leu Val Lys Glu Gly Arg Leu
     50                  55                  60

Glu Glu Glu Val Ala Leu Lys Ile Ile Asn Asp Gly Ala Ala Ile Leu
 65                  70                  75                  80

Arg Gln Glu Lys Thr Met Ile Glu Val Asp Ala Pro Ile Thr Val Cys
                 85                  90                  95

Gly Asp Ile His Gly Gln Phe Phe Asp Leu Met Lys Leu Phe Glu Val
            100                 105                 110

Gly Gly Ser Pro Ser Asn Thr Arg Tyr Leu Phe Leu Gly Asp Tyr Val
        115                 120                 125

Asp Arg Gly Tyr Phe Ser Ile Glu Cys Val Leu Tyr Leu Trp Ser Leu
    130                 135                 140

Lys Ile Asn His Pro Lys Thr Leu Phe Leu Leu Arg Gly Asn His Glu
145                 150                 155                 160

Cys Arg His Leu Thr Asp Tyr Phe Thr Phe Lys Gln Glu Cys Arg Ile
                165                 170                 175

Lys Tyr Ser Glu Gln Val Tyr Asp Ala Cys Met Glu Thr Phe Asp Cys
            180                 185                 190

Leu Pro Leu Ala Ala Leu Leu Asn Gln Gln Phe Leu Cys Val His Gly
        195                 200                 205

Gly Met Ser Pro Glu Ile Thr Ser Leu Asp Asp Ile Arg Lys Leu Asp
    210                 215                 220

Arg Phe Thr Glu Pro Pro Ala Phe Gly Pro Val Cys Asp Leu Leu Trp
225                 230                 235                 240

Ser Asp Pro Ser Glu Asp Tyr Gly Asn Glu Lys Thr Leu Glu His Tyr
                245                 250                 255

Thr His Asn Thr Val Arg Gly Cys Ser Tyr Phe Tyr Ser Tyr Pro Ala
            260                 265                 270

Val Cys Glu Phe Leu Gln Asn Asn Asn Leu Leu Ser Ile Ile Arg Ala
        275                 280                 285

His Glu Ala Gln Asp Ala Gly Tyr Arg Met Tyr Arg Lys Ser Gln Ala
    290                 295                 300

Thr Gly Phe Pro Ser Leu Ile Thr Ile Phe Ser Ala Pro Asn Tyr Leu
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305                 310                 315                 320

Asp Val Tyr Asn Asn Lys Ala Ala Val Leu Lys Tyr Glu Asn Asn Val
                325                 330                 335

Met Asn Ile Arg Gln Phe Asn Cys Ser Pro His Pro Tyr Trp Leu Pro
            340                 345                 350

Asn Phe Met Asp Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys
        355                 360                 365

Val Thr Glu Met Leu Val Asn Val Leu Asn Ile Cys Ser Asp Asp Glu
    370                 375                 380

Leu Ile Ser Asp Asp Glu Ala Glu Gly Ser Thr Thr Val Arg Lys Glu
385                 390                 395                 400

Ile Ile Arg Asn Lys Ile Arg Ala Ile Gly Lys Met Ala Arg Val Phe
                405                 410                 415

Ser Ile Leu Arg Gln Glu Ser Glu Ser Val Leu Thr Leu Lys Gly Leu
            420                 425                 430

Thr Pro Thr Gly Thr Leu Pro Leu Gly Val Leu Ser Gly Gly Lys Gln
        435                 440                 445

Thr Ile Glu Thr Ala Lys Gln Glu Ala Ala Glu Glu Arg Glu Ala Ile
    450                 455                 460

Arg Gly Phe Ser Leu Gln His Lys Ile Arg Ser Phe Glu Glu Ala Arg
465                 470                 475                 480

Gly Leu Asp Arg Ile Asn Glu Arg Met Pro Pro Arg Lys Asp Ser Ile
                485                 490                 495

Tyr Pro Gly Gly Pro Met Lys Ser Val Thr Ser Ala His Ser His Ala
            500                 505                 510

Ala His Arg Ser Asp Gln Gly Lys Lys Ala His Ser
        515                 520
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<210> 34
<211> 170
<212> PRT
<213> Homo sapiens

<400> 34

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Met Gly Asn Glu Ala Ser Tyr Pro Leu Glu Met Cys Ser His Phe Asp
  1                 5                  10                  15

Ala Asp Glu Ile Lys Arg Leu Gly Lys Arg Phe Lys Lys Leu Asp Leu
            20                  25                  30

Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Leu Pro Glu
        35                  40                  45

Leu Gln Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
    50                  55                  60

Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val Ser
65                  70                  75                  80
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```
Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
                 85                  90                  95

Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
            100                 105                 110

Phe Gln Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
        115                 120                 125

Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Gly
    130                 135                 140

Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly Leu
145                 150                 155                 160

Asp Ile His Lys Lys Met Val Val Asp Val
                165                 170
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<210> 35
<211> 19
<212> PRT
<213> Bos taurus

<220>
<221> PEPTIDE
<222> (1)..(19)
<223> peptide of cAMP regulated protein kinase A, RII

<220>
<221> MOD_RES
<222> (15)
<223> PHOSPHORYLATION

<220>
<221> SITE
<222> (15)
<223> artificial chemical modification by
fluoroscein-phosphoramidite

<400> 35

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Asp Leu Asp Val Pro Ile Pro Gly Arg Phe Asp Arg Arg Val Ser Val
  1               5                  10                  15

Ala Ala Glu
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